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Are Drosophila-preferences for yeasts stable or contextual?

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Complete List of Authors:	Günther, Catrin S; University of Lincoln College of Science, School of Life Sciences; Crown Research Institutes Knight, Sarah; The University of Auckland, School of biological Sciences Jones, Rory; University of Lincoln College of Science, School of Life Sciences Goddard, Matthew; University of Lincoln College of Science, School of Life Sciences; The University of Auckland, School of biological Sciences
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ECE-2018-10-01218 "Are Drosophila-preferences for yeasts stable or contextual?"

Response to Editors:

I would like to thank the editors for their patience and support with our manuscript submitted to Ecology and Evolution. We have addressed the raised concerns in the current revision #2 which we are pleased to submit for consideration to be published and made available to the scientific community. Please refer to detailed responses as listed below and highlighted as track changes in the manuscript. Again, I would like to thank you for your interest in our study and especially for the constructive comments on the manuscript.

Sincerely

Catrin S Günther

Comments to the Author:

The authors have done a good job addressing the reviewer concerns with two small exceptions.

CG*: Thank you very much. The comments were helpful in improving the clarity of the manuscript.*

1. The authors need to explicitly address in their manuscript that the nature of the "heritable" change observed in the selection experiments is unknown and could be genetic, epigenetic, or due to shifts in commensal microbiota.

CG: *This is an important aspect have missed to emphasize and we have addressed this now in revision #2 L455-458: "Although the nature of this heritable change in yeast-preference is unknown, these data reject the hypothesis that at least D. simulans yeast preference is conserved. It suggests that selection may operate and readily move this trait, possibly by affecting sensitivity to particular YVOCs which and could be genetic, epigenetic, or due to shifts in commensal microbiota."*

2. Sample spectra for the reported MS peaks must be provided in some public venue (in addition to the provided retention time). This the only way that different studies using MS can begin to find commonalities. This is especially important when no chemical standards were run. The scientific community must be given the opportunity to independently assess the chemical ID assigned to a given MS profile. The expectation of publication of spectra is well established within the metabolomics community and the challenge of extracting data from commercial software does not negate the obligation of making the data public. It is probably easiest to provide this data as a standard tab delimited text file.

CG: *Thank you for pointing this out. I was not previously aware of open source software (such as OpenChrom) which enables conversion of Shimadzu data files (qgd.) into readable formats. I have used this software in conjunction with NIST17 MS search V. 2.2 to match respective sample spectra to the NIST Library. Matches of sample spectra (from strawberry ferments) with NIST17 annotations are now provided in Supplemental material B in a head-to tail format for all compounds / peaks listed in Table 1. We have removed any additional tentative compound identifications from the manuscript and associated materials as these are not relevant to analysis/results and discussions. I feel that this appendix will enable the reader to independently evaluate validity of compound identifications.*

Please refer to the highlighted changes in the Track-changed version of rev#2 with particular attention to deletions. L346-350; L366-368 and reclassification of previously 2,3 – pentanedione and 2-methyl-3-hexanol to unknown 1 and 2, respectively as identities could not be confirmed with NIST17 (previous version NIST 05).

Original Research Article

Are *Drosophila*-preferences for yeasts stable or contextual?

Catrin S. Günther*[1], Sarah J. Knight[2], Rory Jones[1] and Matthew R. Goddard[1,2]

[1]School of Life Sciences, University of Lincoln, Joseph Banks Laboratories, Green Lane, Lincoln, LN6 7DL, United Kingdom

and

[2]School of Biological Sciences, The University of Auckland, New Zealand

*Corresponding author: Catrin S. Günther

Current address: The New Zealand Institute for Plant and Food Research Ltd, 120 Mt Albert Road, Auckland 1025, New Zealand

Catrin.Guenther@plantandfood.co.nz

ABSTRACT

Whether there are general mechanisms driving inter-specific chemical communication is uncertain. *Saccharomycetaceae* yeast and *Drosophila* fruit flies, both extensively studied research models, share the same fruit habitat and it has been suggested their interaction comprises a facultative mutualism that is instigated and maintained by yeast volatiles. Using choice-tests, experimental evolution and volatile analyses we investigate the maintenance of this relationship and reveal little consistency between behavioural responses of two isolates of sympatric *Drosophila* species. While *D. melanogaster* was attracted to a range of different *Saccharomycetaceae* yeasts and this was independent of fruit type, *D. simulans* preference appeared specific to a particular *S. cerevisiae* genotype isolated from a vineyard fly population. This response, however, was not consistent across fruit-types and is therefore context-dependent. In addition, *D. simulans* attraction to an individual *S. cerevisiae* isolate was pliable over ecological time-scales. Volatile candidates were analysed to identify a common signal for yeast attraction, and while *D. melanogaster* generally responded to fermentation profiles, *D. simulans* preference was more discerning and likely threshold-dependent. Overall there is no strong evidence to support the idea of bespoke interactions with specific yeasts for either of these *Drosophila* genotypes. Rather the data support the idea *Drosophila* are generally adapted to sense and locate fruits infested by a range of fungal microbes and/or that yeast-*Drosophila* interactions may evolve rapidly.

KEYWORDS: Chemical communication; *Drosophila*; fruit; mutualism; *Saccharomycetaceae;* yeast

INTRODUCTION

Chemical communication between organisms is ancient and regulates a variety of important intraspecific (Venuleo et al., 2017, Leonhardt et al., 2016) and interspecific biological interactions within ecological networks (Archie and Theis, 2011, Pickett and Khan, 2016). Behavioural responses to olfactory stimuli can be both learned and intrinsic (Bergstrom, 2008), and natural selection may operate on traits that are heritable and correlate with fitness for both signal sender and receiver (West et al., 2007). Pheromones are a classic example of intra-specific chemical signals that communicate social behaviour beneficial to members of the same species (Yew and Chung, 2017, Leonhardt et al., 2016). Other volatile compounds serve to repulse predators (Deletre et al., 2016) and thus elevate fitness by decreasing inter-species interactions. Finally, some volatile blends, such as floral scents, may act as inter-species attractants where both the sender and receiver mutually benefit from the chemically-mediated information (Schiestl, 2010, Raguso, 2008). It has been suggested that chemical signals evolve from unintentional cues (Steiger et al., 2011, Weiss et al., 2013), but these are often defined by complex blends of volatiles rather than single key compounds, and the volatiles implicated might have multiple biological functions (Kessler et al., 2013, Tan and Nishida, 2012). However, once chemically-mediated facultative mutualistic interactions between species are established, it is not clear how robust they are nor how they evolve (Buser et al., 2014). This means we are unable to predict whether all members of a species show the same extent of behavioural response to the same chemical cues and thus have no understanding of how stable or fluctuating these interactions are in nature.

As it stands, we are unable to predict the extent of variance in the efficacy of communication and thus interaction between members of facultative interacting species: are there differences in chemosensory preference or perception within species? How similarly do closely related

species sense and respond equally to mutualistic partners? Overall we have no understanding of how stable or changeable chemically-mediated facultative mutualistic interactions are in nature.

The role of fungal volatiles as semiochemicals attracting insects is well described (Beck and Vannette, 2017, Madden et al., 2018) and *Saccharomycetaceae* (budding) yeasts and *Drosophila* flies in the 'melanogaster' subgroup (Clark et al., 2007) are not only influential research models but also co-inhabit economically important fruit crops (Hamby et al., 2012, Lam and Howell, 2015) where certain *Drosophila* species (such as *D. suzukii*) may act as nuisance and damaging pests (Walsh et al., 2011), and yeasts may variously have negative, benign or positive impacts on fruits or their fermented products (Gschaedler, 2017, Suh et al., 2006). While a variety of *Saccharomycetaceae* yeast species are found associated with fruits (Masneuf-Pomarede et al., 2016, Taylor et al., 2014) they are also found in a range of other niches (Morrison-Whittle et al., 2017, Gayevskiy and Goddard, 2016). There are approximately twenty genera in the *Saccharomycetaceae* family, and most tend to be associated with the early fermentation of fruits (Masneuf-Pomarede et al., 2016, Suh et al., 2006) and *Saccharomyces cerevisiae* and *S. uvarum* tend to dominate from mid-ferment on (Marsit and Dequin, 2015). The available data show these yeast species display significant genetic and geographic diversity (Gayevskiy and Goddard, 2016). There are well over 1,600 *Drosophila* species, which also have large genetic and geographic diversity (O'Grady and DeSalle, 2018), but of these, *D. melanogaster* is the most studied. At least *D. melanogaster* olfactorial pathways appear tuned to microbial volatiles (Mansourian and Stensmyr, 2015), and these volatiles influence behavioural decisions for substrates selected for food and oviposition (Becher et al., 2012, Stokl et al., 2010).

While *Drosophila* in the *melanogaster* subgroup breed in fruit, they derive an array of fitness benefits from consuming yeasts which include influences on sexual receptivity (Gorter et al., 2016), fecundity and larvae development (Buser et al., 2014, Rohlfs and Kurschner, 2010) and other life history traits (Anagnostou et al., 2010). It is therefore unsurprising that at least *D. melanogaster* and *D. simulans* are strongly attracted to certain yeast derived volatiles (Becher et al., 2012, Buser et al., 2014, Stokl et al., 2010, Gunther et al., 2015, Madden et al., 2018). Yeasts metabolise fruit precursors to produce energy and biomass, but also release a range of yeast volatile organic compounds (YVOCs) as they do so (Hazelwood et al., 2008, Cordente et al., 2012). Yeasts are immotile and thus doomed to local extinction along with ephemeral fruits they inhabit. Logically, traits which increase the propensity of at least some members of a yeast colony to be transported to new habitats, which they may then colonise, will be under positive selection (Madden et al., 2018, Christiaens et al., 2014). Following this hypothesis, one *S. cerevisiae* isolate (ScNZ) has been shown to derive fitness benefits from interacting with an isofemale *D. simulans* population, indicating this interaction might comprise a mutualism (Buser et al., 2014). However, there is evidence that other *S. cerevisiae* isolates, as well as isolates from other *Saccharomycetaceae* species, are repulsive to some *Drosophila* (Palanca et al., 2013, Buser et al., 2014), and so it is not yet clear how general or specific any mutualism might be (Günther, 2018). Yeasts produce ethanol which has been shown to induce interference competition with microbes (Goddard, 2008), but the function of most YVOCs, if indeed they have any other than representing stochastic metabolic endpoints, is not at all well understood (Saerens et al., 2010). Here we use the fungi-fruit-fly system as a model to test how robust facultative chemically-mediated inter-species interactions are. Using preference testing, experimental evolution and volatile analysis we ask:

1) Does yeast preference differ between Drosophila genotypes?

D. simulans and *D. melanogaster* are closely related (Clark et al., 2007, O'Grady and DeSalle, 2018) sympatric (Capy and Gibert, 2004) and attracted to banana and commercial (Vector 960) traps and to the yeast-like chemical mimicry of the Solomon's lily (Stokl et al., 2010), suggesting similar chemosensory preferences in both species. However, we have previously shown that isolates from *D. simulans* and *D. melanogaster* are variably attracted to *S. cerevisiae* yeast genotypes grown in grape juice (Gunther et al., 2015, Palanca et al., 2013). Whether there are specific yeast-fly pairings in which *Saccharomycetaceae* yeasts are consistently attractive to *Drosophila* is not clear.

2) Does the fruit context modulate yeast preference?

Drosophila attraction appears contingent on a blend of YVOCs and fruit-derived compounds (Cordente et al., 2012) suggesting any yeast-fly associations should be considered as part of a tripartite relationship including fruits/plants. However, the impact of the fruit component on the putative yeast-fly association has received little attention. Studies testing host plant specificity of cactophilic *D. mojavensis* show that host plant-preference can shift in response to plant-microbe and also microbe-microbe interactions (Date et al., 2017). However, it is not known whether different fruit substrates alter the mode of any yeast-fly interaction, and thus the degree to which the past and future evolution of yeast-fly interactions are affected by the plant host.

3) Are yeast preferences plastic or conserved?

Virtually nothing is known about the capacity for selection to operate on and change facultative chemically-mediated interactions, though at least two studies show within yeast species variance for fly attraction (Palanca et al., 2013, Buser et al., 2014), suggesting attraction may

potentially evolve quickly by acting on standing variance. The magnitude of potential selective effects on fly attraction will determine the likely stability of these interactions through time.

4) Are there conserved chemical mechanisms underlying Drosophila attraction to yeast?

Plants provide precursors in the form of sugars and amino acids for YVOCs formation. In addition to conversion of glucose to ethanol, a range of other YVOCs are produced, e.g. fusel-alcohols, such as 3-methyl butanol (Hazelwood et al., 2008), and their corresponding acetate esters (ie ethyl acetate and 3-methylbutyl acetate) have been suggested to mediate fruit fly attraction (Gunther et al., 2015, Christiaens et al., 2014) and a core set of YVOCS has been proposed to act as key compounds for *D. melanogaster* attraction (Becher et al., 2012). However, the chemical preference of *D. simulans* was not driven simply by the presence or absence of these compounds but involved more subtle combinations of relative ratios of YVOCs in combination with a suite of fruit-derived background odours (Gunther et al., 2015). Single lines of both, *D. simulans* and *D. melanogaster*, were previously described as mutualistic partners for chemically-mediated dispersal of *S. cerevisiae* (Buser et al., 2014, Christiaens et al., 2014). It is however not clear whether *Drosophila* attraction is mediated by a universal YVOCs signal or whether chemically-mediated yeast preference is plastic and contextual and thus might arise by chance (Günther, 2018).

METHODS

Fruit juice ferments

Fresh fruit was obtained from fresh-produce markets and local farms around Lincoln (Galley Hill farm, UK), rinsed with sterile water, and juiced with a sterilised (Distel Laboratory Surface Disinfectant) kitchen juicer (Braun J-500). Clarified juice was sterilised with dimethyl dicarbonate (1:2500, Sigma-Aldrich) and stored at -80°C. Juice sterility was confirmed by

spread-plating on YPDA (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) and Nutrient agar (Fisher bioreagents) and 5-days at 28°C and 35°C, respectively. Replicate (n=6) samples of yeast isolates were inoculated into 5 mL of juice at $5x10^6$ cells per mL and incubated for 48h at 28°C and 200 rpm. To test for variation in attraction to different yeasts, eleven isolates consisting of eight different fruit or insect associated genera were inoculated in strawberry juice (supplemental Table SMM). In an earlier study, *S. cerevisiae* strain FlyKR_78.3 (ScNZ) was isolated from *D. simulans* and found to be attractive when inoculated in Sauvignon Blanc grape juice (Gunther et al., 2015, Buser et al., 2014), whereas *S. cerevisiae* DBVP6044 (ScWA; Liti et al., 2009) was shown to be repulsive. To test for the impact of the fruit context on yeast preference attractiveness of these two *S. cerevisiae* isolates was compared when inoculated in plum (var. 'Victoria'), apple (var. 'Jonagold') and strawberry juice. Total soluble solids were measured with a PAL-1 refractometer (ATAGO™) before and after incubation to evaluate sugar consumption as proxy for fermentation progress. The supernatant was frozen at -80°C for volatile profiling and behavioural assays.

Behavioural study

We used an isofemale *Drosophila simulans* line derived from a vineyard population near Auckland, New Zealand (Buser et al., 2014), and the standard *D. melanogaster* Oregon R wild-type (Carolina®). Both fly species were propagated at 25°C and 12:12 light:dark cycle as described earlier (Gunther et al., 2015). Starved (25 h) females (n=80; 3-7 days old) were used in two-way T-maze choice tests (30 min in dark, 6 h before dark cycle, n=6) with 10 mL (1:1000 dilution) of each ferment and sterile juice as control (Palanca et al., 2013, Buser et al., 2014, Gunther et al., 2015). Head-to-head competition experiments between ScNZ and ScWA were also performed in each fruit type in order to compare against preference to sterile juice. Flies were anaesthetised on ice for 5-min before entering the T-maze and euthanized after the

experiment at -20°C. An attraction index (AI) was calculated (Buser et al., 2014) using the proportion of flies found in either arm of the T-maze, and the binominal distribution was used to test whether the dispersal of flies between both arms of the T-maze was significantly different from random.

Drosophila experimental evolution

The ancestral *D. simulans* population derived from a single female that was isolated from a wild population (see above) and propagated in the lab for 3-years using the conditions above before the experiment. Nine populations were founded by approximately 20 females each from the base population and were evolved over a six months period equating to ten *Drosophila* generations per treatment (Fig 1): 1) control populations, where no selection for fly choice was applied; 2) selection for flies choosing a yeast strain that was attractive to the founding ancestral fly population in strawberry (ScNZ, AI: 0.12; P=0.02); and 3) selection for flies choosing a yeast strain that was significantly less preferred by the founding ancestral fly population (ScWA, AI: -0.05, P=0.01 against ScNZ in strawberry). Each treatment comprised triplicate populations. Selection for fly preference was applied in duplicate per population (N=6 per treatment) and generation with 60-80 female flies per replicate and using all flies (N=30-60) that made the appropriate choice in head-to-head choice tests between ScNZ and ScWA to found the next generation. Control lines were subject to the same head-to-head choices except with sterile juice in both arms and where choice toward one side was chosen randomly to found the next generation. The first emerging progeny (3-7 days old) of each generation were used for selection and consecutive propagation as described. According to choice, female flies were immediately transferred to fresh media for oviposition and killed after three days. Selection wasconducted in the same batch of strawberry juice, and yeasts were not allowed to co-evolve with flies. The eleventh generation of flies from each population was exposed to head-to-head

choice tests using the ancestrally attractive and unattractive yeasts (N=6 per population/ N=18 per treatment) and AIs were calculated. Change in fly preference (AI) during experimental evolution was evaluated by analysis of variance (ANOVA) and Tukey-Kramer Honestly Significantly Difference (Tukey- HSD) corrected post-hoc tests.

Quantitative HS-SPME GC-MS

Volatile profiles were analysed from fruit juice and cell-free ferments using static Headspace Solidphase-Microextraction (HS-SPME) and Gas Chromatography–Mass Spectrometry (GC-MS). The diluted and salt-saturated (1.5g NaCl) sample (4 mL) was mixed with 10 uL 3-heptanol (5mg/L dissolved in water:ethanol 1:1) as internal standard in a 10 mL headspace vial with PTFE seal. Volatiles were extracted from equilibrated samples (30 min, 40°C) for 20 min at 40°C without agitation using a 50/30 µm DVB-Car-PDMS coated fibre (Supelco, 57348-U). Compounds were separated via non-polar GC using a 5-MS column and desorbed thermally using a linear GC-program (40°C hold for 2 min, then 5°C/ min to 200°C and 30°C/min to 300°C) and fragmented in a Quadrupole-MS. Total Ion Chromatogram peaks were selected when: 1) absent in the negative control (water) which was sampled alongside each experiment; 2) present/identifiable in at least three of the six biological replicates; and 3) the spectrum was matched with confidence to compounds from the NIST17library. Peaks were then annotated and automatically detected, identified and integrated using LabSolutions GC-MS Software (Shimadzu Corporation 1999-2006). The identity of compounds was determined by comparing spectra and retention times between different runs and integration was adjusted manually where necessary. Compound names should be regarded as indicative as these were not verified using authentic standards. Compound levels were analysed semi-quantitatively in equivalence to the internal standard (standardised peak area, SPA). Initially, a dilution series was prepared from

one replicate per sample to assess the nature of compounds collected from the headspace, their probability of identification, peak separation and to approximate linearity and limit of quantification. An appropriate dilution (strawberry 1:8, plum 1:8, apple 1:16) was determined for each fruit type based on this initial dilution series yielding reasonable linearity ($R^2 > 0.9$) for the majority of compounds. Where correlation of the SPA to sample dilution was not possible, volatiles were recorded for presence/absence only.

Statistical analyses of volatile profiles

Chemical diversity was calculated following Simpson's diversity index for proportional data (Hill, 1973) and analysed using non-parametric tests (Kruskal-Wallis followed by Mann-Whitney U for post-hoc analysis, (α=0.05)). Differences between fruit types, yeast genotypes and fly attraction were analysed using a subset of volatiles based on their presence in inoculated fruit of at least two different fruit types. The effects of fruit type and yeast genotype on the chemical composition of the ferments were tested with PERMANOVA as implemented in the R package *vegan* (Anderson, 2001), using Jaccard distances and 10,000 permutations. Constrained Correspondence Analysis (CCA), was used for data visualisation using the full factorial model of *fruit type* x *yeast genotype*. The contribution of each chemical was independently investigated using a full factorial ANOVA design of *fruit type* x *yeast genotype*, including an adjustment for multiple tests (Benjamini and Hochberg method; (Benjamini and Hochberg, 1995). A random forest analysis (Breiman, 2001) was performed using the R package *randomForest* (Liaw, 2002) to identify which compounds correlated with fly attraction and generated bootstrapped regression trees based on the explanatory variables (chemical concentrations) and estimates how important each variable is in explaining the response (AI, fly attraction, treated as a continuous variable). Correlation tests between each individual chemical and the attraction indices of both *D. simulans* and *D. melanogaster* were

performed using Pearson's product moment correlation coefficient and the P-values were adjusted for multiple tests (Benjamini and Hochberg, 1995). Chemical compounds that were identified as correlating with fly attraction were partitioned using conditional inference tree analysis as implemented in the R package *party* (Horthorn, 2006). Conditional inference trees create binary partitions in the data based on statistically significant differences, minimising bias and over-fitting.

RESULTS

1) Contrasting yeast preferences in *Drosophila* lines suggest that *D. melanogaster* is likely attracted to a broad range of *Saccharomycetaceae* whereas *D. simulans* response appears species specific. To test whether there are specific yeast preferences between genotypes of *Drosophila* subgroup *'melanogaster'* fly lines, we measured the AI of eleven fruit or insect derived *Saccharomycetaceae* yeast isolates (supplemental Table SMM) grown in strawberry juice using binary choice tests. The *D. melanogaster* line was significantly attracted to eight of the eleven yeasts isolates (Fig 2) spanning *Hanseniaspora uvarum, H. occidentalis*, *Candida zemplininia, C. apicola* and *S. cerevisiae* ($P<0.02$, Fig 2). There was no choice preference for *S. uvarum* ($P=0.09$) or the *Pichia* isolates ($P>0.4$), and none of the yeast isolates were repulsive to *D. melanogaster*. Using the same yeast ferments, the *D. simulans* line was significantly attracted to just one *S. cerevisiae* strain (ScNZ, $P=2x10^{-4}$): the same strain previously reported as attractive to this same *D. simulans* line when grown in grape juice (Gunther et al., 2015, Buser et al., 2014). However, in contrast to *D. melanogaster*, *D. simulans* was indifferent to most other yeasts, including other *S. cerevisiae* strains, and repulsed by the *Pichia kluyverii* ($P=0.02$) and *P. pijperi* ($P=1x10^{-4}$) isolates (Fig 2). Overall only one yeast strain was consistently significantly attractive to both fly species (ScNZ), and thus these results support

that yeast preference differs, at least between the *Drosophila* genotypes derived from closely related species tested here.

2) Yeast attraction was stable across fruit-types for *D. melanogaster* but *D. simulans* showed context dependent behaviour. The attraction of both *Drosophila* lines to two *S. cerevisiae* isolates (ScNZ and ScWA (Gunther et al., 2015, Buser et al., 2014)) when grown in strawberry, plum and apple juice showed that this *D. melanogaster* genotype was significantly attracted to both yeast isolates in all fruits ($P<7.2 \times 10^{-5}$; Fig 3A) compared to sterile juice. In contrast, whether yeasts were attractive to the *D. simulans* genotype was contingent on the fruit context (Fig 3B). For example, *D. simulans* was attracted to ScNZ in strawberry juice ($P=2 \times 10^{-4}$), but not in plum ($P= 0.5$). This same line has previously been shown to be repulsed by ScWA in grape (Gunther et al., 2015), and here ScWA had a negative AI in strawberry (AI: -0.08, $P=0.12$), but was significantly attractive in plum ($P=0.002$) and apple ($P=0.01$). Thus, yeast preference of this *D. simulans* genotype was heavily dependent on the fruit environment. Competition (head-to-head) comparisons between both yeasts in the various fruits confirmed that *D. melanogaster* was equally attracted to both isolates across fruits except strawberry where ScWA was preferred over ScNZ ($P=1.2 \times 10^{-5}$, Fig 3A). Overall these data support a lack of fruit effect for *D. melanogaster* attraction to yeasts and suggest that any resulting interactions are likely broad and stable. For the *D. simulans* isolate however, these data indicate that the fruit context significantly affects whether a particular yeast isolate is attractive and therefore any resulting yeast-fly interactions are contingent on both fruit and yeast type, and thus are not generally yeast-type specific.

3) *Drosophila* Yeast-preferences evolve over ecological time frame. Whether *Drosophila* attraction to particular yeasts is conserved or malleable was examined using experimental

evolution of isogenic *D. simulans* over a six-month period with initially attractive (ScNZ, AI: 0.2; *P*=0.0002) and less-preferred yeast (ScWA, AI: -0.05, *P*=0.01 in head-to-head competition with ScNZ, Fig 3b). There was no change in fly preference in the control lines compared to the ancestor (*P*=0.55, supplemental A, Table SA2), showing the experimental system the flies were subjected to had no effect on their yeast preference. However, there were significant differences in fly preferences between the ancestor and lines subject to selection (ANOVA, *F*-ratio = 5.3, *P*=0.009) as shown in Fig 4. Tukey-HSD post-hoc analysis (α=0.05) show lines selected for attraction to the originally preferential yeast strain (ScNZ) retained this attraction (P = 0.87, TableSA3). However, fly lines selected for preference to the originally less preferred yeast (ScWA) evolved to become significantly attracted to ScWA (P=0.02, Fig 3, supplemental Table SA3). Thus, *D. simulans* preference significantly changed over a relatively short time-scale to become attracted to the originally less-preferable yeast strain, with the trade-off of decreased preference to the originally attractive yeast.

4) **A universal chemical signal driving yeast attraction across *Drosophila* genotypes is unlikely.** Thus far the data show that yeast-fly interactions are not conserved between the *Drosophila* lines tested here and may change between fruit contexts and over time. However, *Drosophila* is able to recognise some component/s of YVOCs and this induces a behavioural response. We tested whether there was a general chemical signal mediating *Drosophila* attraction. Between 41 and 60 volatile compounds were tentatively identified in sterile plum, apple and strawberry juice using GC-MS, with only 17 compounds common to all fruits. Thus, the majority of volatiles were fruit-type specific. Fermentation significantly reduced the number of volatiles from an average of 53±6 to 42±4 (P=0.02) but increased profile similarity across different fruits from 13.5% to 68%. The volatile composition between ferments of different fruits had greater similarity to one another than to the sterile juices of those fruits

(strawberry: 5.1%; plum: 12.1%; apple: 10.2%). Consequently, fermentation significantly ($P<0.001$) affected the volatile profile of each fruit type by reducing chemical diversity compared to juice (supplemental SC1, Fig SC1) and increasing the abundance of single compounds. While it is well known that yeast metabolism alters the composition and concentrations of fruit volatiles (Cordente et al., 2012), we show that the yeast manipulated chemical signatures of ferments from different fruits tend to converge.

The *D. melanogaster* lab strain was highly attracted to *S. cerevisiae* ferments regardless of yeast isolate or fruit type, and thus one might conclude that compared to sterile juice, YVOCs unique or predominant in these ferments comprise a common signal and drive attraction. Forty-two compounds were common in these fruit types and respective ferments and were grouped into those that were: 1) exclusive to juice; 2) exclusive to ferments; and 3) present in both but significantly greater in ferments (Table 1). We found that seven volatiles were indicative of sterile juice. These compounds do not correlate with and thus unlikely represent drivers of fly attraction. Twenty six compounds were defined as YVOCs as these were either exclusive to or consistently and significantly ($P<0.03$) increased in ferments across all samples (Table 1, supplemental B). Of these, 2-methylbutanol, 3-methylbutanol and 3-methylbutyl acetate showed a strong interaction between fruit and yeast type (ANOVA with FDR, supplemental Table SD1) indicating that different yeast isolates are metabolising fruit precursors in different ways. Of those compounds that did not show a significant interaction, unknown volatile 1 and 2, , linalool and β-damascenone were differentiated between fruit types, but not yeast types, and 1-heptanol was differentiated between yeast but not fruit types (supplemental Table SC1).

A clear separation between fruit type (Fig 5B) and yeast genotype (Fig 5C) was visualised using CCA, and three compounds (3-hexenol, 2-methyl propanol and unknown 2) had loadings

(Eigenvectors * √Eigenvalues) with the highest magnitude in the analysis (Fig 5A), thus appearing to drive variation between samples.

An alternative approach to analyse the multivariate chemical profiles uses PermANOVA and this confirmed that volatile profiles were affected by both yeast (R^2=9%, P=10^{-4}) and fruit type (R^2=19%, P=10^{-4}, supplemental Table SE2). However, the interaction between fruit and yeast type explained 27% (P=10^{-4}) of the total variation indicating, in line with the preceding approach, that YVOCs are the likely product of a complex, metabolic interaction between different fruits and yeasts.

The correlation between volatile profiles and fly attraction was further analysed by integrating the AI's (supplemental Table SE1) with the volatile data using a random forest analysis. Analyses of both *D. simulans* and *D. melanogaster* returned best models where the predicted AI of each fly was negatively correlated with the observed AI, indicating models were not able to identify a significant effect of single compounds on fly attraction. After adjusting the *P*-values for multiple tests, there were no significant correlations with individual compounds and AI (supplemental Table SE1). This shows that single compounds are not strongly implicated in fly attraction. However, relying on P-values solely may be misleading particularly when multiple tests are performed (Krzywinski and Altman, 2014). To further interrogate the data, we went on to use conditional inference tree analysis: for *D. simulans*, significant binary splits were made for ethyl acetate, 3-methyl butanol and 2-methyl butanol (supplemental Fig SF1), implicating these in attraction. Greater ethyl acetate and 2-methyl butanol concentrations correlate with a greater attraction response ($P = 0.04$ and $P = 0.01$) for *D. simulans* as part of a blend when present above defined thresholds. When inoculated in strawberry juice, the levels of six YVOCs were significantly (Mann-Whitney U-test, $P<0.05$, supplemental Table SC4)

increased in the preferred yeast ScNZ compared to ScWA ferments and these included ethyl acetate, 3-methyl butanol, 2-methyl butanol besides 2-methyl propanol, hexyl acetate and 2-phenyl ethanol. For *D. melanogaster*, a significant binary split could be made for 2-methylpropyl acetate, a compound that also differs between fruit-type. Here attraction decreased with greater 2-methylpropyl acetate levels suggesting a repulsive effect above thresholds (supplemental Fig SF2). Given that Conditional Interference trees was the only test that showed any significant result, further experiments would be required to verify these findings. Overall there is no compelling data to support the hypothesis that specific yeast volatiles might act as general signal driving attraction of *Drosophila*. While the data confirm an array of YVOCs as candidates for attraction of the *D. melanogaster* line, flies from the *D. simulans* population were likely to respond in a context-specific and threshold-dependent way.

DISCUSSION

If selection has been and is operating on chemically-mediated interactions between *Drosophila* and *Saccharomycetaceae* yeasts then one predicts specific yeast genotypes to be consistently attractive (or repulsive). The data categorically reject this general hypothesis, with only one (ScNZ) of eleven yeast isolates demonstrating consistency in attractiveness to both *Drosophila* tested. Instead the data show *Drosophila*-genotype specific profiles where *D. simulans* was indifferent to, and *D. melanogaster* was attracted to, the majority of yeast isolates (Fig 2). This in-turn suggests that either specific yeast- preferences evolved *de novo* for each of these isolates, or any intrinsic response inherited from the common *D. simulans*/*melanogaster* ancestor to have differentially evolved.

Spatial or temporal resource partitioning is a commonly described mechanism promoting coexistence of species using the same nutritional resource (Chesson, 2000). For example, the Drosophilids *Zaprionus indianus* and *D. simulans* coexist in figs by colonising the fruit at

different ripening stages and larval dietary requirements of both species correspond to increasing yeast infestation during fruit ripening (Matavelli et al., 2015). This raises the question whether differentiation in chemosensory preference might promote the coexistence of closely related species. *D. simulans* and *D. melanogaster* are evolutionary siblings that split about 2.5 million years ago (Clark et al., 2007), form hybrids and live in sympatry. Possible mechanisms regulating their coexistence were mainly explained by adaptation strategies leading to seasonal variation in life history traits and differences in their ecophysiology (Capy and Gibert, 2004, David et al., 2004). Some studies also report increased ethanol and concomitant acetic acid sensitivity in *D. simulans* (Chakir et al., 1993). However, the degree to which differential preferences for microbial volatiles may have impacted resource selection and thus niche differentiation between the two species has to our knowledge not been previously investigated, and these data suggest this possibility. The caveat to this conclusion is that single lines of flies were used – it would be valuable to understand the variance in attraction among different fly genotypes in *D. simulans*/*melanogaster* generally.

Following the logic introduced above, if selection has been and is operating on YVOC-mediated specific *Drosophila* attraction then maintenance of any associated traits would be expected regardless of environmental factors like the fruit context. The data support this hypothesis for the *D. melanogaster* line where both *S. cerevisiae* isolates were significantly attractive in all three fruits (Fig 3A) but not for *D. simulans*' yeast preference which was contingent on the fruit host. This shows the fruit context modulates yeast attraction in one fly line only, and that response again differs between *Drosophila* genotypes which provides further support that resulting yeast-*Drosophila* interactions may evolve readily.

We tested whether this trait was movable or stable directly, using experimental evolution with *D. simulans* and show that fly preference significantly changed over ten generations to become attracted to an originally less-preferable yeast (Fig 4). The generation time of *Drosophila* in nature is not clear, but the ten generations/six months in controlled conditions covered in this experiment likely represent at least a summer season in the wild. Thus, the attraction of the *D. simulans* isolate to specific yeasts appears to be plastic and capable of fluctuating over short ecologically representative time periods, even within members from an isogenic female population. Although the nature of this heritable change is unknown, these data reject the hypothesis that at least *D. simulans* yeast preference is conserved. It suggests that selection may operate and readily move this trait, possibly by affecting sensitivity to particular YVOCs which and could be genetic, epigenetic, or due to shifts in commensal microbiota. This is a third line of evidence providing support that yeast-*Drosophila* interactions are potentially capable of rapidly evolving.

That yeast fermentation changes volatile profiles of fruits is well known. However, it is surprising to find such a high degree of volatile homogeneity across ferments from vastly different horticultural crops, indicating a universal chemical signal for the presence of yeasts. The majority of these YVOCs were tentatively classified as esters (in particular ethyl and acetate esters), fusel-alcohols and other intermediates of the Ehrlich-pathway (Günther, 2018, Hazelwood et al., 2008). In particular C_2/C_3 substituted or branched-chained volatiles likely resulting from amino acid assimilation were abundant. *Drosophila* odour receptors are known to respond to these metabolites, in particular 3-hydroxy-2-butanone, 2-phenyl ethanol, 2-phenethyl acetate, 3-methyl butanol and 3-methylbutyl acetate were previously suggested to act as key compounds for chemical attraction (Becher et al., 2012, Arguello et al., 2013, Stokl et al., 2010, Gunther et al., 2015), and it is likely these volatiles are involved in driving

attraction in *D. melanogaster*. However, *D. simulans'* attraction was sensitive to the fruit, threshold and context dependent. It appears that flies might respond to structurally related compounds rather than to individual ones but what defines these mechanisms or drives them apart between species is yet to be elucidated.

Overall this suite of experiments strongly suggests that chemically mediated interactions differ at least between individual lines of *D. melanogaster*/*D. simulans* and *Saccharomycetaceae* yeasts. Further, the strength and nature of the *D. simulans*-yeast attraction may change over relatively short ecological time scales. At the least, these data strongly suggest that the evolution of chemosensory perception differs between two *Drosophila* genotypes in the *melanogaster* sub-group, and thus supports a conclusion that there is no ancient or 'fixed' aspect to *Drosophila* (sub-group *melanogaster)* - yeast interaction that selection has maintained. The data do support the conclusion that the association between some *Drosophila* lines (in this case *D. melanogaster)* and *Saccharomycetaceae* yeasts is relatively stable. However, the chemical mechanisms of this interaction remain elusive. Overall there is no strong evidence to support the idea that there are bespoke interactions with specific yeast species or strains for either of these organisms, rather the data support the idea that *Drosophila* are generally adapted to sense and locate fruits infested by a range of fungal microbes, as would be expected in nature. While particular experiments with inter-species interactions may appear to conform to mutualisms, it is perhaps necessary to understand the variation of these traits among populations and species to fully substantiate any claims as to whether natural selection has produced adaptations for mutualistic interactions.

We postulate these interactions may be due to two possible reasons. Firstly, they may be due to exaptation (selection operated on yeast volatile production for other reasons), and that flies

have evolved to sense YVOC's to prey on yeasts, in which case specific yeast transfer between fruits by *Drosophila* and other insects is nothing greater than an fortuitous event, and not one driven by natural selection (Günther, 2018). Alternatively, the ecological reality is that microbes exist as communities (a mix of different individuals and species) in/on fruit (Taylor et al., 2014), and the concept of a single strain of any microbe metabolising and emitting volatiles in isolation seems highly unlikely. It is possible that selection may instead operate on a higher-level 'community bouquet'. Here selection operates on fly attraction to YVOCs produced by more general yeast (and possibly bacterial) infected fruits, and that this microbial community is then generally dispersed to other fruits. This hypothesis predicts that mixes of yeast strains and species will be more attractive than individual isolates, and that selection will have operated to assemble a core set of species that comprise a fruit microbiome.

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AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: CSG, MRG. Performed the experiments: CSG, RJ. Analysed the data: CSG, SJK, RJ, MG. Wrote the manuscript: CSG, SJK, MRG.

STATEMENT ABOUT COMPETING INTERESTS

The authors declare no competing financial or non-financial interests.

DATA ACCESSIBILITY STATEMENT

The datasets generated and/or analysed during the current study will be made publicly available through the University of Lincoln Data repository upon acceptance of the submitted manuscript.

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FIGURE AND TABLE CAPTIONS

Figure 1: Mechanism of selection for female *Drosophila simulans* flies (Ds) with preference to an initially attractive *Saccharomyces cerevisiae* strain (ScNZ) and initially unattractive strain (ScWA) when inoculated in strawberry juice. . Triplicate populations were founded for each treatment from the ancestral population and were evolved over a six month period equating to

ten *Drosophila* generations. The treatments were: 1) control populations (DsC), where no selection for fly choice was applied: the next generation was founded from flies selected from one side of the T-maze at random; 2) selection for flies choosing the yeast strain that was attractive to the founding ancestral fly population (ScNZ) and these are labelled DsNZ; and 3) selection for flies choosing the yeast strain (ScWA) that was significantly less preferred by the founding ancestral fly population, and these populations are labelled DsWA. Selection for fly preference was applied each generation by head-to-head competition between ScNZ and ScWA with 60-80 female flies per replicate population in duplicate. According to choice, 32-70 flies per population were transferred to fresh media for oviposition and killed after three days.

Figure 2: *Drosophila melanogaster* and *D. simulans* attraction to a range of *Saccharomycetaceae* yeasts grown in strawberry juice. Error bars represent the standard error of the mean attraction index and significance in binomial distribution of choice tests is indicated by asterisks (N=6, α=0.05).

Figure 3: Attractiveness of *Saccharomyces cerevisiae* strains ScNZ and ScWA to *Drosophila melanogaster* (A) and *D. simulans* (B) when inoculated in sterile fruit juice and compared against juice of the same fruit type as control. Binary competition experiments (ScNZ/ScWA) test ferments of both yeasts against each other, where a positive Attraction index (AI) indicates preference for ScNZ and a negative AI preference for ScWA. Error bars represent the standard error of the mean AI and significance in binomial distribution of choice tests is indicated by asterisks (N=6, α=0.05).

Figure 4: Change in *Drosophila simulans* preference to *S. cerevisiae* isolates ScNZ and ScWA in strawberry juice over ten fly generations. Error bars represent the standard error of the mean attraction index (AI, N=6) for ancestral *D. simulans* and lines selected for attraction to ScNZ (DsNZ) or ScWA (DsWA), respectively. Positive AI indicate preference for ScNZ and negative AI attraction to ScWA and treatments not connected by the same letter are significantly different (ANOVA, Tukey HSD test at α=0.05).

Figure 5: Constrained Correspondence Analysis visualisation of fruit ferments using the chemicals listed in Table 1. (A) The direction and magnitude of all loading vectors, with labels for chemicals that report a magnitude larger than 0.5. The blue circles represent the position of 0.1, 0.25, 0.5 and 1 for reference. (B) Sample points coloured by fruit type with 50% ellipses. (C) Samples point coloured by yeast genotype with 50% ellipses.

Table 1: Volatiles common to sterile strawberry (**S**), apple (**A**) and plum (**P**) juice and to juice inoculated with *Saccharomyces cerevisiae* strains ScNZ and ScWA, respectively. Grouping indicates volatiles that were significantly increased and decreased (*P<0.05, ***P<0.005; multivariate analysis of variance, Tukey HSD corrected) in juice compared to ferments. ° indicates that compound levels were outside the quantification limit and only considered for qualitative analysis.

Original Research Article

Are *Drosophila*-preferences for yeasts stable or contextual?

Catrin S. Günther*[1], Sarah J. Knight[2], Rory Jones[1] and Matthew R. Goddard[1,2]

[1]School of Life Sciences, University of Lincoln, Joseph Banks Laboratories, Green Lane, Lincoln, LN6 7DL, United Kingdom

and

[2]School of Biological Sciences, The University of Auckland, New Zealand

*Corresponding author: Catrin S. Günther

Current address: The New Zealand Institute for Plant and Food Research Ltd, 120 Mt Albert Road, Auckland 1025, New Zealand

Catrin.Guenther@plantandfood.co.nz

ABSTRACT

Whether there are general mechanisms driving inter-specific chemical communication is uncertain. *Saccharomycetaceae* yeast and *Drosophila* fruit flies, both extensively studied research models, share the same fruit habitat and it has been suggested their interaction comprises a facultative mutualism that is instigated and maintained by yeast volatiles. Using choice-tests, experimental evolution and volatile analyses we investigate the maintenance of this relationship and reveal little consistency between behavioural responses of two isolates of sympatric *Drosophila* species. While *D. melanogaster* was attracted to a range of different *Saccharomycetaceae* yeasts and this was independent of fruit type, *D. simulans* preference appeared specific to a particular *S. cerevisiae* genotype isolated from a vineyard fly population. This response, however, was not consistent across fruit-types and is therefore context-dependent. In addition, *D. simulans* attraction to an individual *S. cerevisiae* isolate was pliable over ecological time-scales. Volatile candidates were analysed to identify a common signal for yeast attraction, and while *D. melanogaster* generally responded to fermentation profiles, *D. simulans* preference was more discerning and likely threshold-dependent. Overall there is no strong evidence to support the idea of bespoke interactions with specific yeasts for either of these *Drosophila* genotypes. Rather the data support the idea *Drosophila* are generally adapted to sense and locate fruits infested by a range of fungal microbes and/or that yeast-*Drosophila* interactions may evolve rapidly.

KEYWORDS: Chemical communication; *Drosophila*; fruit; mutualism; *Saccharomycetaceae;* yeast

INTRODUCTION

Chemical communication between organisms is ancient and regulates a variety of important intraspecific (Venuleo et al., 2017, Leonhardt et al., 2016) and interspecific biological interactions within ecological networks (Archie and Theis, 2011, Pickett and Khan, 2016). Behavioural responses to olfactory stimuli can be both learned and intrinsic (Bergström, 2008), and natural selection may operate on traits that are heritable and correlate with fitness for both signal sender and receiver (West et al., 2007). Pheromones are a classic example of intra-specific chemical signals that communicate social behaviour beneficial to members of the same species (Yew and Chung, 2017, Leonhardt et al., 2016). Other volatile compounds serve to repulse predators (Deletre et al., 2016) and thus elevate fitness by decreasing inter-species interactions. Finally, some volatile blends, such as floral scents, may act as inter-species attractants where both the sender and receiver mutually benefit from the chemically-mediated information (Schiestl, 2010, Raguso, 2008). It has been suggested that chemical signals evolve from unintentional cues (Steiger et al., 2011, Weiss et al., 2013), but these are often defined by complex blends of volatiles rather than single key compounds, and the volatiles implicated might have multiple biological functions (Kessler et al., 2013, Tan and Nishida, 2012). However, once chemically-mediated facultative mutualistic interactions between species are established, it is not clear how robust they are nor how they evolve (Buser et al., 2014). This means we are unable to predict whether all members of a species show the same extent of behavioural response to the same chemical cues and thus have no understanding of how stable or fluctuating these interactions are in nature.

As it stands, we are unable to predict the extent of variance in the efficacy of communication and thus interaction between members of facultative interacting species: are there differences in chemosensory preference or perception within species? How similarly do closely related

species sense and respond equally to mutualistic partners? Overall we have no understanding of how stable or changeable chemically-mediated facultative mutualistic interactions are in nature.

The role of fungal volatiles as semiochemicals attracting insects is well described (Beck and Vannette, 2017, Madden et al., 2018) and *Saccharomycetaceae* (budding) yeasts and *Drosophila* flies in the 'melanogaster' subgroup (Clark et al., 2007) are not only influential research models but also co-inhabit economically important fruit crops (Hamby et al., 2012, Lam and Howell, 2015) where certain *Drosophila* species (such as *D. suzukii*) may act as nuisance and damaging pests (Walsh et al., 2011), and yeasts may variously have negative, benign or positive impacts on fruits or their fermented products (Gschaedler, 2017, Suh et al., 2006). While a variety of *Saccharomycetaceae* yeast species are found associated with fruits (Masneuf-Pomarede et al., 2016, Taylor et al., 2014) they are also found in a range of other niches (Morrison-Whittle et al., 2017, Gayevskiy and Goddard, 2016). There are approximately twenty genera in the *Saccharomycetaceae* family, and most tend to be associated with the early fermentation of fruits (Masneuf-Pomarede et al., 2016, Suh et al., 2006) and *Saccharomyces cerevisiae* and *S. uvarum* tend to dominate from mid-ferment on (Marsit and Dequin, 2015). The available data show these yeast species display significant genetic and geographic diversity (Gayevskiy and Goddard, 2016). There are well over 1,600 *Drosophila* species, which also have large genetic and geographic diversity (O'Grady and DeSalle, 2018), but of these, *D. melanogaster* is the most studied. At least *D. melanogaster* olfactorial pathways appear tuned to microbial volatiles (Mansourian and Stensmyr, 2015), and these volatiles influence behavioural decisions for substrates selected for food and oviposition (Becher et al., 2012, Stökl et al., 2010).

While *Drosophila* in the *melanogaster* subgroup breed in fruit, they derive an array of fitness benefits from consuming yeasts which include influences on sexual receptivity (Gorter et al., 2016), fecundity and larvae development (Buser et al., 2014, Rohlfs and Kürschner, 2010) and other life history traits (Anagnostou et al., 2010). It is therefore unsurprising that at least *D. melanogaster* and *D. simulans* are strongly attracted to certain yeast derived volatiles (Becher et al., 2012, Buser et al., 2014, Stökl et al., 2010, Günther et al., 2015, Madden et al., 2018). Yeasts metabolise fruit precursors to produce energy and biomass, but also release a range of yeast volatile organic compounds (YVOCs) as they do so (Hazelwood et al., 2008, Cordente et al., 2012). Yeasts are immotile and thus doomed to local extinction along with ephemeral fruits they inhabit. Logically, traits which increase the propensity of at least some members of a yeast colony to be transported to new habitats, which they may then colonise, will be under positive selection (Madden et al., 2018, Christiaens et al., 2014). Following this hypothesis, one *S. cerevisiae* isolate (ScNZ) has been shown to derive fitness benefits from interacting with an isofemale *D. simulans* population, indicating this interaction might comprise a mutualism (Buser et al., 2014). However, there is evidence that other *S. cerevisiae* isolates, as well as isolates from other *Saccharomycetaceae* species, are repulsive to some *Drosophila* (Palanca et al., 2013, Buser et al., 2014), and so it is not yet clear how general or specific any mutualism might be (Günther & Goddard, 2019). Yeasts produce ethanol which has been shown to induce interference competition with microbes (Goddard, 2008), but the function of most YVOCs, if indeed they have any other than representing stochastic metabolic endpoints, is not at all well understood (Saerens et al., 2010). Here we use the fungi-fruit-fly system as a model to test how robust facultative chemically-mediated inter-species interactions are. Using preference testing, experimental evolution and volatile analysis we ask:

1) Does yeast preference differ between Drosophila genotypes?

D. simulans and *D. melanogaster* are closely related (Clark et al., 2007, O'Grady and DeSalle, 2018) sympatric (Capy and Gibert, 2004) and attracted to banana and commercial (Vector 960) traps and to the yeast-like chemical mimicry of the Solomon's lily (Stökl et al., 2010), suggesting similar chemosensory preferences in both species. However, we have previously shown that isolates from *D. simulans* and *D. melanogaster* are variably attracted to *S. cerevisiae* yeast genotypes grown in grape juice (Günther et al., 2015, Palanca et al., 2013). Whether there are specific yeast-fly pairings in which *Saccharomycetaceae* yeasts are consistently attractive to *Drosophila* is not clear.

2) Does the fruit context modulate yeast preference?

Drosophila attraction appears contingent on a blend of YVOCs and fruit-derived compounds (Cordente et al., 2012) suggesting any yeast-fly associations should be considered as part of a tripartite relationship including fruits/plants. However, the impact of the fruit component on the putative yeast-fly association has received little attention. Studies testing host plant specificity of cactophilic *D. mojavensis* show that host plant-preference can shift in response to plant-microbe and also microbe-microbe interactions (Date et al., 2017). However, it is not known whether different fruit substrates alter the mode of any yeast-fly interaction, and thus the degree to which the past and future evolution of yeast-fly interactions are affected by the plant host.

3) Are yeast preferences plastic or conserved?

Virtually nothing is known about the capacity for selection to operate on and change facultative chemically-mediated interactions, though at least two studies show within yeast species variance for fly attraction (Palanca et al., 2013, Buser et al., 2014), suggesting attraction may

potentially evolve quickly by acting on standing variance. The magnitude of potential selective effects on fly attraction will determine the likely stability of these interactions through time.

4) Are there conserved chemical mechanisms underlying Drosophila attraction to yeast?

Plants provide precursors in the form of sugars and amino acids for YVOCs formation. In addition to conversion of glucose to ethanol, a range of other YVOCs are produced, e.g. fusel-alcohols, such as 3-methyl butanol (Hazelwood et al., 2008), and their corresponding acetate esters (ie ethyl acetate and 3-methylbutyl acetate) have been suggested to mediate fruit fly attraction (Günther et al., 2015, Christiaens et al., 2014) and a core set of YVOCS has been proposed to act as key compounds for *D. melanogaster* attraction (Becher et al., 2012). However, the chemical preference of *D. simulans* was not driven simply by the presence or absence of these compounds but involved more subtle combinations of relative ratios of YVOCs in combination with a suite of fruit-derived background odours (Günther et al., 2015). Single lines of both, *D. simulans* and *D. melanogaster*, were previously described as mutualistic partners for chemically-mediated dispersal of *S. cerevisiae* (Buser et al., 2014, Christiaens et al., 2014). It is however not clear whether *Drosophila* attraction is mediated by a universal YVOCs signal or whether chemically-mediated yeast preference is plastic and contextual and thus might arise by chance (Günther & Goddard, 2019).

METHODS

Fruit juice ferments

Fresh fruit was obtained from fresh-produce markets and local farms around Lincoln (Galley Hill farm, UK), rinsed with sterile water, and juiced with a sterilised (Distel Laboratory Surface Disinfectant) kitchen juicer (Braun J-500). Clarified juice was sterilised with dimethyl dicarbonate (1:2500, Sigma-Aldrich) and stored at -80°C. Juice sterility was confirmed by

spread-plating on YPDA (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) and Nutrient agar (Fisher bioreagents) and 5-days at 28°C and 35°C, respectively. Replicate (n=6) samples of yeast isolates were inoculated into 5 mL of juice at $5x10^6$ cells per mL and incubated for 48h at 28°C and 200 rpm. To test for variation in attraction to different yeasts, eleven isolates consisting of eight different fruit or insect associated genera were inoculated in strawberry juice (supplemental Table SMM). In an earlier study, *S. cerevisiae* strain FlyKR_78.3 (ScNZ) was isolated from *D. simulans* and found to be attractive when inoculated in Sauvignon Blanc grape juice (Günther et al., 2015, Buser et al., 2014), whereas *S. cerevisiae* DBVP6044 (ScWA; Liti et al., 2009) was shown to be repulsive. To test for the impact of the fruit context on yeast preference attractiveness of these two *S. cerevisiae* isolates was compared when inoculated in plum (var. 'Victoria'), apple (var. 'Jonagold') and strawberry juice. Total soluble solids were measured with a PAL-1 refractometer (ATAGO™) before and after incubation to evaluate sugar consumption as proxy for fermentation progress. The supernatant was frozen at -80°C for volatile profiling and behavioural assays.

Behavioural study

We used an isofemale *Drosophila simulans* line derived from a vineyard population near Auckland, New Zealand (Buser et al., 2014), and the standard *D. melanogaster* Oregon R wild-type (Carolina®). Both fly species were propagated at 25°C and 12:12 light:dark cycle as described earlier (Günther et al., 2015). Starved (25 h) females (n=80; 3-7 days old) were used in two-way T-maze choice tests (30 min in dark, 6 h before dark cycle, n=6) with 10 mL (1:1000 dilution) of each ferment and sterile juice as control (Palanca et al., 2013, Buser et al., 2014, Günther et al., 2015). Head-to-head competition experiments between ScNZ and ScWA were also performed in each fruit type in order to compare against preference to sterile juice. Flies were anaesthetised on ice for 5-min before entering the T-maze and euthanized after the

experiment at -20°C. An attraction index (AI) was calculated (Buser et al., 2014) using the proportion of flies found in either arm of the T-maze, and the binominal distribution was used to test whether the dispersal of flies between both arms of the T-maze was significantly different from random.

Drosophila experimental evolution

The ancestral *D. simulans* population derived from a single female that was isolated from a wild population (see above) and propagated in the lab for 3-years using the conditions above before the experiment. Nine populations were founded by approximately 20 females each from the base population and were evolved over a six months period equating to ten *Drosophila* generations per treatment (Fig 1): 1) control populations, where no selection for fly choice was applied; 2) selection for flies choosing a yeast strain that was attractive to the founding ancestral fly population in strawberry (ScNZ, AI: 0.12; P=0.02); and 3) selection for flies choosing a yeast strain that was significantly less preferred by the founding ancestral fly population (ScWA, AI: -0.05, P=0.01 against ScNZ in strawberry). Each treatment comprised triplicate populations. Selection for fly preference was applied in duplicate per population (N=6 per treatment) and generation with 60-80 female flies per replicate and using all flies (N=30-60) that made the appropriate choice in head-to-head choice tests between ScNZ and ScWA to found the next generation. Control lines were subject to the same head-to-head choices except with sterile juice in both arms and where choice toward one side was chosen randomly to found the next generation. The first emerging progeny (3-7 days old) of each generation were used for selection and consecutive propagation as described. According to choice, female flies were immediately transferred to fresh media for oviposition and killed after three days. Selection wasconducted in the same batch of strawberry juice, and yeasts were not allowed to co-evolve with flies. The eleventh generation of flies from each population was exposed to head-to-head

choice tests using the ancestrally attractive and unattractive yeasts (N=6 per population/ N=18 per treatment) and AIs were calculated. Change in fly preference (AI) during experimental evolution was evaluated by analysis of variance (ANOVA) and Tukey-Kramer Honestly Significantly Difference (Tukey- HSD) corrected post-hoc tests.

Quantitative HS-SPME GC-MS

Volatile profiles were analysed from fruit juice and cell-free ferments using static Headspace Solidphase-Microextraction (HS-SPME) and Gas Chromatography–Mass Spectrometry (GC-MS). The diluted and salt-saturated (1.5g NaCl) sample (4 mL) was mixed with 10 uL 3-heptanol (5mg/L dissolved in water:ethanol 1:1) as internal standard in a 10 mL headspace vial with PTFE seal. Volatiles were extracted from equilibrated samples (30 min, 40°C) for 20 min at 40°C without agitation using a 50/30 µm DVB-Car-PDMS coated fibre (Supelco, 57348-U). Compounds were separated via non-polar GC using a 5-MS column and desorbed thermally using a linear GC-program (40°C hold for 2 min, then 5°C/ min to 200°C and 30°C/min to 300°C) and fragmented in a Quadrupole-MS. Total Ion Chromatogram peaks were selected when: 1) absent in the negative control (water) which was sampled alongside each experiment; 2) present/identifiable in at least three of the six biological replicates; and 3) the spectrum was matched with confidence ~~high probability (>990)~~ to compounds from the NIST17~~ist05~~ library. Peaks were then annotated and automatically detected, identified and integrated using LabSolutions GC-MS Software (Shimadzu Corporation 1999-2006). The identity of compounds was determined by comparing spectra ~~to the NIST05 library~~ and retention times between different runs and integration was adjusted manually where necessary. Compound names should be regarded as indicative as these were not verified using authentic standards. Compound levels were analysed semi-quantitatively in equivalence to the internal standard

(standardised peak area, SPA). Initially, a dilution series was prepared from one replicate per sample to assess the nature of compounds collected from the headspace, their probability of identification, peak separation and to approximate linearity and limit of quantification. An appropriate dilution (strawberry 1:8, plum 1:8, apple 1:16) was determined for each fruit type based on this initial dilution series yielding reasonable linearity ($R^2 > 0.9$) for the majority of compounds. Where correlation of the SPA to sample dilution was not possible, volatiles were recorded for presence/absence only.

Statistical analyses of volatile profiles

Chemical diversity was calculated following Simpson's diversity index for proportional data (Hill, 1973) and analysed using non-parametric tests (Kruskal-Wallis followed by Mann-Whitney U for post-hoc analysis, (α=0.05)). Differences between fruit types, yeast genotypes and fly attraction were analysed using a subset of volatiles based on their presence in inoculated fruit of at least two different fruit types. The effects of fruit type and yeast genotype on the chemical composition of the ferments were tested with PERMANOVA as implemented in the R package *vegan* (Anderson, 2001), using Jaccard distances and 10,000 permutations. Constrained Correspondence Analysis (CCA), was used for data visualisation using the full factorial model of *fruit type* x *yeast genotype*. The contribution of each chemical was independently investigated using a full factorial ANOVA design of *fruit type* x *yeast genotype*, including an adjustment for multiple tests (Benjamini and Hochberg method; Benjamini & Hochberg, 1995). A random forest analysis (Breiman, 2001) was performed using the R package *randomForest* (Liaw, 2002) to identify which compounds correlated with fly attraction and generated bootstrapped regression trees based on the explanatory variables (chemical concentrations) and estimates how important each variable is in explaining the response (AI, fly attraction, treated as a continuous variable). Correlation tests between each

individual chemical and the attraction indices of both *D. simulans* and *D. melanogaster* were performed using Pearson's product moment correlation coefficient and the P-values were adjusted for multiple tests (Benjamini & Hochberg, 1995). Chemical compounds that were identified as correlating with fly attraction were partitioned using conditional inference tree analysis as implemented in the R package *party* (Horthorn, 2006). Conditional inference trees create binary partitions in the data based on statistically significant differences, minimising bias and over-fitting.

RESULTS

1) Contrasting yeast preferences in *Drosophila* lines suggest that *D. melanogaster* is likely attracted to a broad range of *Saccharomycetaceae* whereas *D. simulans* response appears species specific. To test whether there are specific yeast preferences between genotypes of *Drosophila* subgroup *'melanogaster'* fly lines, we measured the AI of eleven fruit or insect derived *Saccharomycetaceae* yeast isolates (supplemental Table SMM) grown in strawberry juice using binary choice tests. The *D. melanogaster* line was significantly attracted to eight of the eleven yeasts isolates (Fig 2) spanning *Hanseniaspora uvarum, H. occidentalis*, *Candida zemplininia, C. apicola* and *S. cerevisiae* ($P<0.02$, Fig 2). There was no choice preference for *S. uvarum* ($P=0.09$) or the *Pichia* isolates ($P>0.4$), and none of the yeast isolates were repulsive to *D. melanogaster*. Using the same yeast ferments, the *D. simulans* line was significantly attracted to just one *S. cerevisiae* strain (ScNZ, $P=2x10^{-4}$): the same strain previously reported as attractive to this same *D. simulans* line when grown in grape juice (Günther et al., 2015, Buser et al., 2014). However, in contrast to *D. melanogaster*, *D. simulans* was indifferent to most other yeasts, including other *S. cerevisiae* strains, and repulsed by the *Pichia kluyverii* ($P=0.02$) and *P. pijperi* ($P=1x10^{-4}$) isolates (Fig 2). Overall only one yeast strain was consistently significantly attractive to both fly species (ScNZ), and thus these results support

that yeast preference differs, at least between the *Drosophila* genotypes derived from closely related species tested here.

2) Yeast attraction was stable across fruit-types for *D. melanogaster* but *D. simulans* showed context dependent behaviour. The attraction of both *Drosophila* lines to two *S. cerevisiae* isolates (ScNZ and ScWA (Günther et al., 2015, Buser et al., 2014)) when grown in strawberry, plum and apple juice showed that this *D. melanogaster* genotype was significantly attracted to both yeast isolates in all fruits ($P<7.2 \times 10^{-5}$; Fig 3A) compared to sterile juice. In contrast, whether yeasts were attractive to the *D. simulans* genotype was contingent on the fruit context (Fig 3B). For example, *D. simulans* was attracted to ScNZ in strawberry juice ($P=2 \times 10^{-4}$), but not in plum ($P= 0.5$). This same line has previously been shown to be repulsed by ScWA in grape (Günther et al., 2015), and here ScWA had a negative AI in strawberry (AI: -0.08, $P=0.12$), but was significantly attractive in plum ($P=0.002$) and apple ($P=0.01$). Thus, yeast preference of this *D. simulans* genotype was heavily dependent on the fruit environment. Competition (head-to-head) comparisons between both yeasts in the various fruits confirmed that *D. melanogaster* was equally attracted to both isolates across fruits except strawberry where ScWA was preferred over ScNZ ($P=1.2 \times 10^{-5}$, Fig 3A). Overall these data support a lack of fruit effect for *D. melanogaster* attraction to yeasts and suggest that any resulting interactions are likely broad and stable. For the *D. simulans* isolate however, these data indicate that the fruit context significantly affects whether a particular yeast isolate is attractive and therefore any resulting yeast-fly interactions are contingent on both fruit and yeast type, and thus are not generally yeast-type specific.

3) *Drosophila* Yeast-preferences evolve over ecological time frame. Whether *Drosophila* attraction to particular yeasts is conserved or malleable was examined using experimental

evolution of isogenic *D. simulans* over a six-month period with initially attractive (ScNZ, AI: 0.2; *P*=0.0002) and less-preferred yeast (ScWA, AI: -0.05, *P*=0.01 in head-to-head competition with ScNZ, Fig 3b). There was no change in fly preference in the control lines compared to the ancestor (*P*=0.55, supplemental A, Table SA2), showing the experimental system the flies were subjected to had no effect on their yeast preference. However, there were significant differences in fly preferences between the ancestor and lines subject to selection (ANOVA, *F*-ratio = 5.3, *P*=0.009) as shown in Fig 4. Tukey-HSD post-hoc analysis (α=0.05) show lines selected for attraction to the originally preferential yeast strain (ScNZ) retained this attraction (P = 0.87, TableSA3). However, fly lines selected for preference to the originally less preferred yeast (ScWA) evolved to become significantly attracted to ScWA (P=0.02, Fig 3, supplemental Table SA3). Thus, *D. simulans* preference significantly changed over a relatively short time-scale to become attracted to the originally less-preferable yeast strain, with the trade-off of decreased preference to the originally attractive yeast.

4) **A universal chemical signal driving yeast attraction across *Drosophila* genotypes is unlikely.** Thus far the data show that yeast-fly interactions are not conserved between the *Drosophila* lines tested here and may change between fruit contexts and over time. However, *Drosophila* is able to recognise some component/s of YVOCs and this induces a behavioural response. We tested whether there was a general chemical signal mediating *Drosophila* attraction. Between 41 and 60 volatile compounds were tentatively identified in sterile plum, apple and strawberry juice using GC-MS, with only 17 compounds common to all fruits. Thus, the majority of volatiles were fruit-type specific. ~~, and comprised characteristic chemical classes, such as furans (e.g. Furaneol) in strawberry (Schiefner et al., 2013), aromatic compounds (e.g. phenylacetaldehyde) and terpenes in plum (Pino and Quijano, 2012), and branched chain esters and alcohols in apple (Espino-Díaz et al., 2016) (supplemental Table~~

SB1, Fig SC2). Fermentation significantly reduced the number of volatiles from an average of 53±6 to 42±4 (P=0.02) but increased profile similarity across different fruits from 13.5% to 68%. The volatile composition between ferments of different fruits had greater similarity to one another than to the sterile juices of those fruits (strawberry: 5.1%; plum: 12.1%; apple: 10.2%). Consequently, fermentation significantly (P<0.001) affected the volatile profile of each fruit type by reducing chemical diversity compared to juice (supplemental SC1, Fig SC1) and increasing the abundance of single compounds. While it is well known that yeast metabolism alters the composition and concentrations of fruit volatiles (Cordente et al., 2012), we show that the yeast manipulated chemical signatures of ferments from different fruits tend to converge.

The *D. melanogaster* lab strain was highly attracted to *S. cerevisiae* ferments regardless of yeast isolate or fruit type, and thus one might conclude that compared to sterile juice, YVOCs unique or predominant in these ferments comprise a common signal and drive attraction. Forty-two compounds were common in these fruit types and respective ferments and were grouped into those that were: 1) exclusive to juice; 2) exclusive to ferments; and 3) present in both but significantly greater in ferments (Table 1). We found that seven green leaf volatiles (likely C_6-alcohols and C_6 aldehydes, supplemental Table SC2) that are usually emitted by herbivore-damaged plants (Engelberth et al., 2004) were indicative of sterile juice. These compounds do not correlate with and thus unlikely represent drivers of fly attraction. Twenty six one compounds were defined as YVOCs as these were either exclusive to or consistently and significantly (P<0.03) increased in ferments across all samples (Table 1, supplemental B). Of these, 2-methylbutanol, 3-methylbutanol and 3-methylbutyl acetate showed a strong interaction between fruit and yeast type (ANOVA with FDR, supplemental Table SD1) indicating that different yeast isolates are metabolising fruit precursors in different ways. Of

those compounds that did not show a significant interaction, unknown volatile 1 and 2, ~~2,3-pentanedione, 2-methyl 3-hexanol~~, linalool and β-damascenone were differentiated between fruit types, but not yeast types, and 1-heptanol was differentiated between yeast but not fruit types (supplemental Table SC1).

A clear separation between fruit type (Fig 5B) and yeast genotype (Fig 5C) was visualised using CCA, and three compounds ~~structurally related alcohols~~ (likely 3-hexenol, 2-methyl propanol and ~~unknown 2 2-methyl 3-hexanol~~) had loadings (Eigenvectors * √Eigenvalues) with the highest magnitude in the analysis (Fig 5A), thus appearing to drive variation between samples.

An alternative approach to analyse the multivariate chemical profiles uses PermANOVA and this confirmed that volatile profiles were affected by both yeast (R^2=9%, $P=10^{-4}$) and fruit type (R^2=19%, $P=10^{-4}$, supplemental Table SE2). However, the interaction between fruit and yeast type explained 27% ($P=10^{-4}$) of the total variation indicating, in line with the preceding approach, that YVOCs are the likely product of a complex, metabolic interaction between different fruits and yeasts.

The correlation between volatile profiles and fly attraction was further analysed by integrating the AI's (supplemental Table SE1) with the volatile data using a random forest analysis. Analyses of both *D. simulans* and *D. melanogaster* returned best models where the predicted AI of each fly was negatively correlated with the observed AI, indicating models were not able to identify a significant effect of single compounds on fly attraction. After adjusting the *P*-values for multiple tests, there were no significant correlations with individual compounds and AI (supplemental Table SE1). This shows that single compounds are not strongly implicated

in fly attraction. However, relying on P-values solely may be misleading particularly when multiple tests are performed (Krzywinski and Altman, 2014). To further interrogate the data, we went on to use conditional inference tree analysis: for *D. simulans*, significant binary splits were made for ethyl acetate, 3-methyl butanol and 2-methyl butanol (supplemental Fig SF1), implicating these in attraction. Greater ethyl acetate and 2-methyl butanol concentrations correlate with a greater attraction response ($P = 0.04$ and $P = 0.01$) for *D. simulans* as part of a blend when present above defined thresholds. When inoculated in strawberry juice, the levels of six YVOCs were significantly (Mann-Whitney U-test, $P<0.05$, supplemental Table SC4) increased in the preferred yeast ScNZ compared to ScWA ferments and these included ethyl acetate, 3-methyl butanol, 2-methyl butanol besides 2-methyl propanol, hexyl acetate and 2-phenyl ethanol. For *D. melanogaster*, a significant binary split could be made for 2-methylpropyl acetate, a compound that also differs between fruit-type. Here attraction decreased with greater 2-methylpropyl acetate levels suggesting a repulsive effect above thresholds (supplemental Fig SF2). Given that Conditional Interference trees was the only test that showed any significant result, further experiments would be required to verify these findings. Overall there is no compelling data to support the hypothesis that specific yeast volatiles might act as general signal driving attraction of *Drosophila*. While the data confirm an array of YVOCs as candidates for attraction of the *D. melanogaster* line, flies from the *D. simulans* population were likely to respond in a context-specific and threshold-dependent way.

DISCUSSION

If selection has been and is operating on chemically-mediated interactions between *Drosophila* and *Saccharomycetaceae* yeasts then one predicts specific yeast genotypes to be consistently attractive (or repulsive). The data categorically reject this general hypothesis, with only one (ScNZ) of eleven yeast isolates demonstrating consistency in attractiveness to both *Drosophila*

tested. Instead the data show *Drosophila*-genotype specific profiles where *D. simulans* was indifferent to, and *D. melanogaster* was attracted to, the majority of yeast isolates (Fig 2). This in-turn suggests that either specific yeast- preferences evolved *de novo* for each of these isolates, or any intrinsic response inherited from the common *D. simulans*/*melanogaster* ancestor to have differentially evolved.

Spatial or temporal resource partitioning is a commonly described mechanism promoting coexistence of species using the same nutritional resource (Chesson, 2000). For example, the Drosophilids *Zaprionus indianus* and *D. simulans* coexist in figs by colonising the fruit at different ripening stages and larval dietary requirements of both species correspond to increasing yeast infestation during fruit ripening (Matavelli et al., 2015). This raises the question whether differentiation in chemosensory preference might promote the coexistence of closely related species. *D. simulans* and *D. melanogaster* are evolutionary siblings that split about 2.5 million years ago (Clark et al., 2007), form hybrids and live in sympatry. Possible mechanisms regulating their coexistence were mainly explained by adaptation strategies leading to seasonal variation in life history traits and differences in their ecophysiology (Capy & Gibert, 2004, David et al., 2004). Some studies also report increased ethanol and concomitant acetic acid sensitivity in *D. simulans* (Chakir et al., 1993). However, the degree to which differential preferences for microbial volatiles may have impacted resource selection and thus niche differentiation between the two species has to our knowledge not been previously investigated, and these data suggest this possibility. The caveat to this conclusion is that single lines of flies were used – it would be valuable to understand the variance in attraction among different fly genotypes in *D. simulans*/*melanogaster* generally.

Following the logic introduced above, if selection has been and is operating on YVOC-mediated specific *Drosophila* attraction then maintenance of any associated traits would be

expected regardless of environmental factors like the fruit context. The data support this hypothesis for the *D. melanogaster* line where both *S. cerevisiae* isolates were significantly attractive in all three fruits (Fig 3A) but not for *D. simulans*' yeast preference which was contingent on the fruit host. This shows the fruit context modulates yeast attraction in one fly line only, and that response again differs between *Drosophila* genotypes which provides further support that resulting yeast-*Drosophila* interactions may evolve readily.

We tested whether this trait was movable or stable directly, using experimental evolution with *D. simulans* and show that fly preference significantly changed over ten generations to become attracted to an originally less-preferable yeast (Fig 4). The generation time of *Drosophila* in nature is not clear, but the ten generations/six months in controlled conditions covered in this experiment likely represent at least a summer season in the wild. Thus, the attraction of the *D. simulans* isolate to specific yeasts appears to be plastic and capable of fluctuating over short ecologically representative time periods, even within members from an isogenic female population. Although the nature of this hertitable change in yeast-preference is unknown, these data reject the hypothesis that at least *D. simulans* yeast preference is conserved. It suggests , and show that selection may operate and readily move this trait, possibly by affecting sensitivity to particular YVOCs which and could be genetic, epigenetic, or due to shifts in commensal microbiota. This is a third line of evidence providing support that yeast-*Drosophila* interactions are potentially capable of rapidly evolving.

That yeast fermentation changes volatile profiles of fruits is well known. However, it is surprising to find such a high degree of volatile homogeneity across ferments from vastly different horticultural crops, indicating a universal chemical signal for the presence of yeasts. The majority of these YVOCs were tentatively classified as esters (in particular ethyl and

acetate esters), fusel-alcohols and other intermediates of the Ehrlich-pathway (Günther, 2018, Hazelwood et al., 2008). In particular C_2/C_3 substituted or branched-chained volatiles likely resulting from amino acid assimilation were abundant. *Drosophila* odour receptors are known to respond to these metabolites, in particular 3-hydroxy-2-butanone, 2-phenethyl ethanol, 2-phenethyl acetate, 3-methyl butanol and 3-methylbutyl acetate were previously suggested to act as key compounds for chemical attraction (Becher et al., 2012, Arguello et al., 2013, Stökl et al., 2010, Günther et al., 2015), and it is likely these volatiles are involved in driving attraction in *D. melanogaster*. However, *D. simulans'* attraction was sensitive to the fruit, threshold and context dependent. It appears that flies might respond to structurally related compounds rather than to individual ones but what defines these mechanisms or drives them apart between species is yet to be elucidated.

Overall this suite of experiments strongly suggests that chemically mediated interactions differ at least between individual lines of *D. melanogaster*/*D. simulans* and *Saccharomycetaceae* yeasts. Further, the strength and nature of the *D. simulans*-yeast attraction may change over relatively short ecological time scales. At the least, these data strongly suggest that the evolution of chemosensory perception differs between two *Drosophila* genotypes in the *melanogaster* sub-group, and thus supports a conclusion that there is no ancient or 'fixed' aspect to *Drosophila* (sub-group *melanogaster)* - yeast interaction that selection has maintained. The data do support the conclusion that the association between some *Drosophila* lines (in this case *D. melanogaster)* and *Saccharomycetaceae* yeasts is relatively stable. However, the chemical mechanisms of this interaction remain elusive. Overall there is no strong evidence to support the idea that there are bespoke interactions with specific yeast species or strains for either of these organisms, rather the data support the idea that *Drosophila* are generally adapted to sense and locate fruits infested by a range of fungal microbes, as would

be expected in nature. While particular experiments with inter-species interactions may appear to conform to mutualisms, it is perhaps necessary to understand the variation of these traits among populations and species to fully substantiate any claims as to whether natural selection has produced adaptations for mutualistic interactions.

We postulate these interactions may be due to two possible reasons. Firstly, they may be due to exaptation (selection operated on yeast volatile production for other reasons), and that flies have evolved to sense YVOC's to prey on yeasts, in which case specific yeast transfer between fruits by *Drosophila* and other insects is nothing greater than an fortuitous event, and not one driven by natural selection (Günther & Goddard, 2019). Alternatively, the ecological reality is that microbes exist as communities (a mix of different individuals and species) in/on fruit (Taylor et al., 2014), and the concept of a single strain of any microbe metabolising and emitting volatiles in isolation seems highly unlikely. It is possible that selection may instead operate on a higher-level 'community bouquet'. Here selection operates on fly attraction to YVOCs produced by more general yeast (and possibly bacterial) infected fruits, and that this microbial community is then generally dispersed to other fruits. This hypothesis predicts that mixes of yeast strains and species will be more attractive than individual isolates, and that selection will have operated to assemble a core set of species that comprise a fruit microbiome.

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AUTHORS' CONTRIBUTIONS

Conceived and designed the experiments: CSG, MRG. Performed the experiments: CSG, RJ. Analysed the data: CSG, SJK, RJ, MG. Wrote the manuscript: CSG, SJK, MRG.

STATEMENT ABOUT COMPETING INTERESTS

The authors declare no competing financial or non-financial interests.

DATA ACCESSIBILITY STATEMENT

The datasets generated and/or analysed during the current study will be made publicly available through the University of Lincoln Data repository upon acceptance of the submitted manuscript.

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FIGURE AND TABLE CAPTIONS

Figure 1: Mechanism of selection for female *Drosophila simulans* flies (Ds) with preference to an initially attractive *Saccharomyces cerevisiae* strain (ScNZ) and initially unattractive strain (ScWA) when inoculated in strawberry juice. . Triplicate populations were founded for each

treatment from the ancestral population and were evolved over a six month period equating to ten *Drosophila* generations. The treatments were: 1) control populations (DsC), where no selection for fly choice was applied: the next generation was founded from flies selected from one side of the T-maze at random; 2) selection for flies choosing the yeast strain that was attractive to the founding ancestral fly population (ScNZ) and these are labelled DsNZ; and 3) selection for flies choosing the yeast strain (ScWA) that was significantly less preferred by the founding ancestral fly population, and these populations are labelled DsWA. Selection for fly preference was applied each generation by head-to-head competition between ScNZ and ScWA with 60-80 female flies per replicate population in duplicate. According to choice, 32-70 flies per population were transferred to fresh media for oviposition and killed after three days.

Figure 2: *Drosophila melanogaster* and *D. simulans* attraction to a range of *Saccharomycetaceae* yeasts grown in strawberry juice. Error bars represent the standard error of the mean attraction index and significance in binomial distribution of choice tests is indicated by asterisks (N=6, α=0.05).

Figure 3: Attractiveness of *Saccharomyces cerevisiae* strains ScNZ and ScWA to *Drosophila melanogaster* (A) and *D. simulans* (B) when inoculated in sterile fruit juice and compared against juice of the same fruit type as control. Binary competition experiments (ScNZ/ScWA) test ferments of both yeasts against each other, where a positive Attraction index (AI) indicates preference for ScNZ and a negative AI preference for ScWA. Error bars represent the standard error of the mean AI and significance in binomial distribution of choice tests is indicated by asterisks (N=6, α=0.05).

Figure 4: Change in *Drosophila simulans* preference to *S. cerevisiae* isolates ScNZ and ScWA in strawberry juice over ten fly generations. Error bars represent the standard error of the mean attraction index (AI, N=6) for ancestral *D. simulans* and lines selected for attraction to ScNZ (DsNZ) or ScWA (DsWA), respectively. Positive AI indicate preference for ScNZ and negative AI attraction to ScWA and treatments not connected by the same letter are significantly different (ANOVA, Tukey HSD test at α=0.05).

Figure 5: Constrained Correspondence Analysis visualisation of fruit ferments using the chemicals listed in Table 1. (A) The direction and magnitude of all loading vectors, with labels for chemicals that report a magnitude larger than 0.5. The blue circles represent the position of 0.1, 0.25, 0.5 and 1 for reference. (B) Sample points coloured by fruit type with 50% ellipses. (C) Samples point coloured by yeast genotype with 50% ellipses.

Table 1: Volatiles common to sterile strawberry (**S**), apple (**A**) and plum (**P**) juice and to juice inoculated with *Saccharomyces cerevisiae* strains ScNZ and ScWA, respectively. Grouping indicates volatiles that were significantly increased and decreased (*P<0.05, ***P<0.005; multivariate analysis of variance, Tukey HSD corrected) in juice compared to ferments. ° indicates that compound levels were outside the quantification limit and only considered for qualitative analysis.

***Supplemental material:* "Are *Drosophila*-preferences for yeasts stable or contextual?"**

by Catrin S. Günther, Sarah J. Knight, Rory Jones and Matthew R. Goddard

Table SMM: Origin of *Saccharomycetaceae* isolates.

Name	**Country of origin**	**Source**	**Reference**
Saccharomyces cerevisiae 'Lalvin' EC-1118	France	Commercial wine yeast	Lallemand inc. Lallemandbrewing.com
Saccharomyces cerevisiae Fly_KR78.3	New Zealand	*Drosophila simulans*	Buser et al 2014. Ecology Letters 17: 157-64
Saccharomyces cerevisiae DBVP6044	West Africa	Bili Wine	Liti et al. 2009. Nature 458: 337-41
Saccharomyces uvarum SBJ1d	New Zealand	Sauvignon Blanc ferment	Goddard culture collection
Hanseniaspora uvarum 11-382	USA	*Drosophila suzukii*	Phaff Yeast culture collection, UC-Davis
Hanseniaspora uvarum HB-62	New Zealand	Chardonnay fruit	Gayevskiy et al. 2012. ISME Journal 6:1281-90
Hanseniaspora occidentalis WI-82	New Zealand	Syrah fruit	
Pichia kluyverii JT3.71	New Zealand	Chardonnay juice	Anfang et al. 2009. Australian Journal of Grape and Wine Research 15:1-8
Pichia pijperi	New Zealand	Pinot noir ferment	Goddard culture collection
Candida apicola X120705S2.1	New Zealand	Beehive	Anfang et al. 2009. Australian Journal of Grape and Wine Research 15:1-8
Candida zemplininia FA2.12	New Zealand	Chardonnay ferment	

Supplemental A

Data were analysed using IBM SPSS Statistics Version 21

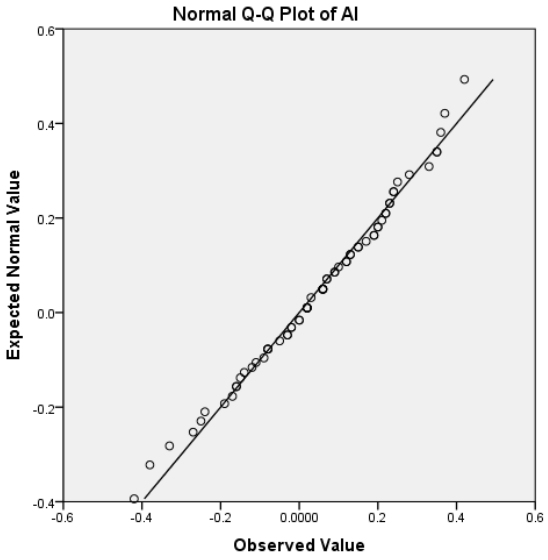


Figure SA1: Q-Q plots indicating roughly normal distribution of combined attraction Indices (AI, N=66) testing *Drosophila simulans* (ancestral line/F0, 3 independent F11 populations) preference for *Saccharomyces cerevisiae*_ ScNZ against *S. cerevisiae* ScWA in two-way choice tests.

TableSA1: Levene's Test of Equality of Error Variances. Tests the null hypothesis of homogeneity of variances in Attraction indices (AI) as the dependent variable across *Drosophila simulans* populations.

F	df1	df2	Sig.
.494	3	62	.687

TableSA2: Paired Samples Test. Testing the null hypothesis that there is no difference in *Drosophila simulans* attraction (AI as dependent variable) for *Saccharomyces cerevisae* ScNZ between the ancestral line (DsF0) and evolved (DsNZF11, DsWAF11) or control/ unevolved offspring population (DsCF11).

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	DsF0 - DsCF11	.05500	.30480	.08799	-.13866	.24866	.625	11	.545
Pair 2	DsF0 - DsNZF11	.07417	.27550	.07953	-.10088	.24921	.933	11	.371
Pair 3	DsF0 - DsWAF11	.22833	.30522	.08811	.03440	.42226	2.591	11	.025

TableSA3: Tukey HSD corrected Multiple Comparisons Testing the null hypothesis that there is no difference in *Drosophila simulans* attraction (AI as dependent variable) for *Saccharomyces cerevisae*_ScNZ between each fly population. DsF0: ancestral line; DsNZF11: evolved to select *S. cerevisiae*_ScNZ; DsWAF11: evolved to select *S. cerevisiae*_ScWA; DsCF11 control/ unevolved offspring

Population (I)	(J) Population	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
DsF0	DsCF11	.0161	.06528	.995	-.1562	.1884
	DsNZF11	.0494	.06528	.873	-.1229	.2218
	DsWAF11	.1989*	.06528	.017	.0266	.3712
DsF11	DsF0	-.0161	.06528	.995	-.1884	.1562
	DsNZF11	.0333	.05838	.940	-.1208	.1875
	DsWAF11	.1828*	.05838	.014	.0286	.3369
DsNZF11	DsF0	-.0494	.06528	.873	-.2218	.1229
	DsCF11	-.0333	.05838	.940	-.1875	.1208
	DsWAF11	.1494	.05838	.061	-.0047	.3036
DsWAF11	DsF0	-.1989*	.06528	.017	-.3712	-.0266
	DsCF11	-.1828*	.05838	.014	-.3369	-.0286
	DsNZF11	-.1494	.05838	.061	-.3036	.0047

Based on observed means.

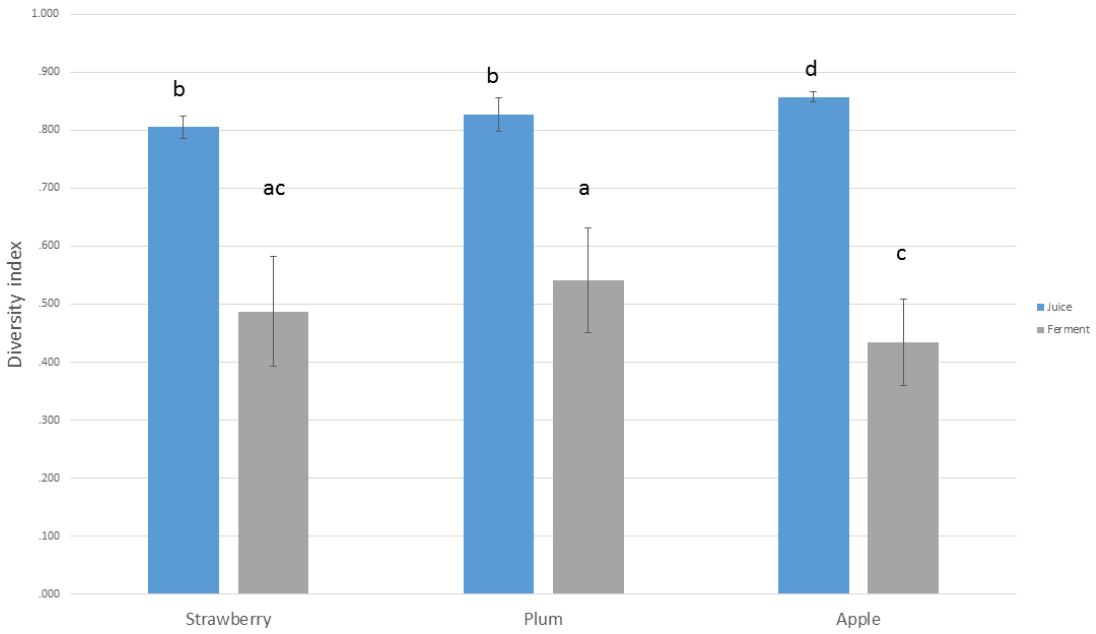
The error term is Mean Square(Error) = .031.

*. The mean difference is significant at the .05 level.

Supplemental C

Table SC1: Number of different peaks, detected in the headspace of sterile and yeast- inoculated juice

Fruit type	sample	N volatiles
Strawberry	Yeast ScNZ	46
	Yeast ScWA	44
	juice	60
Plum	Yeast ScNZ	41
	Yeast ScWA	44
	juice	49
Apple	Yeast ScNZ	35
	Yeast ScWA	41
	juice	51



FigSC1: Chemical diversity of headspace volatiles from inoculated (blue) and sterile (grey) juice. The error bars represent the standard deviation of the mean diversity index (Simpson's diversity) reflecting counts and concentrations of chemical compounds. Kruskal Wallis-H test (X^2=40.4; p<0.0001) indicates a significantly different diversity distribution between samples. This has been confirmed for pairwise comparison using the Mann-Whitney U test, except for samples highlighted with the same letters which are not significantly different from each other in their median chemical diversity.

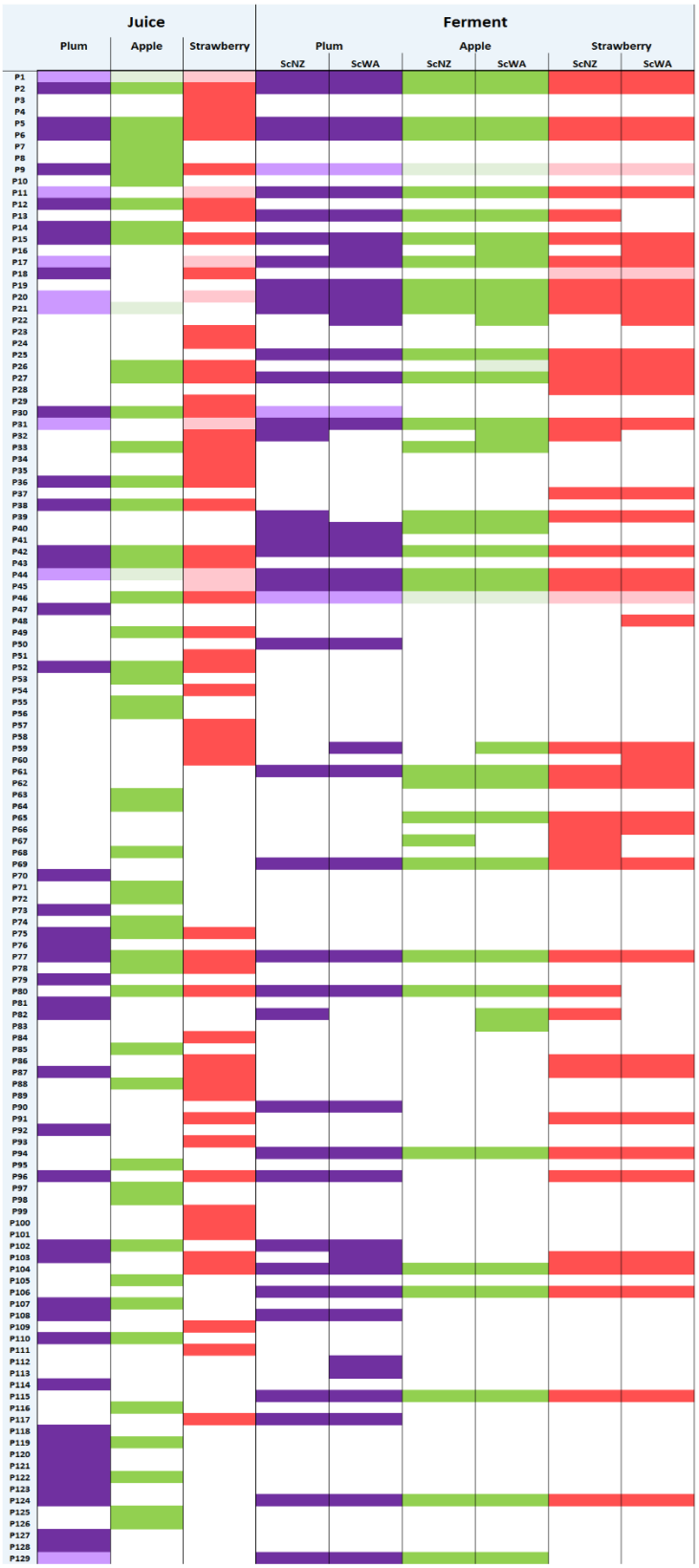


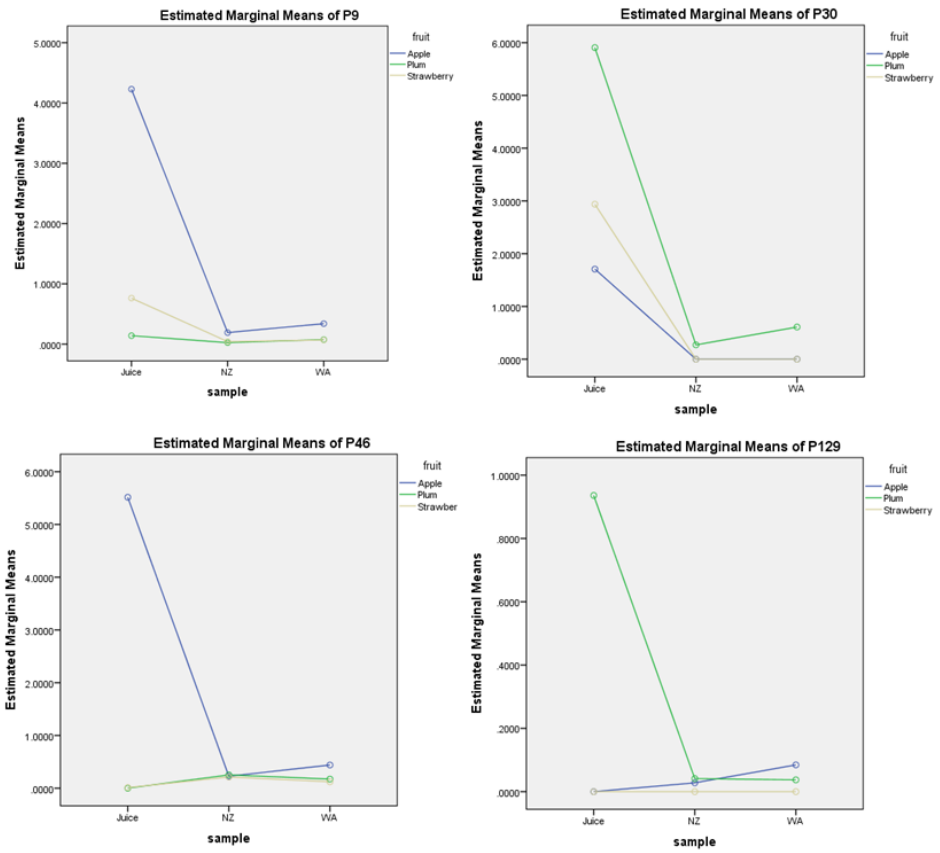
Figure SC2: Volatile profiles of sterile fruit juice and after inoculation with *Saccharomyces cerevisiae* strain ScNZ and ScWA, respectively. Increased colour transparency indicates that compound levels were significantly (P<0.05) lower when compared to the sterile or inoculated sample of the corresponding fruit (TukeyHSD-corrected Manova, see TableSD1 and SD2).

TableSC2: Fruit-specific and yeast specific compounds detected in sterile plum (Pl), apple (Ap) and strawberry (St) juice and after inoculation with *Saccharomyces cerevisiae* ScNZ and *S. cerevisiae* ScWA, respectively. ✓ indicates that the compound is present in a sample and x that is was not detected.

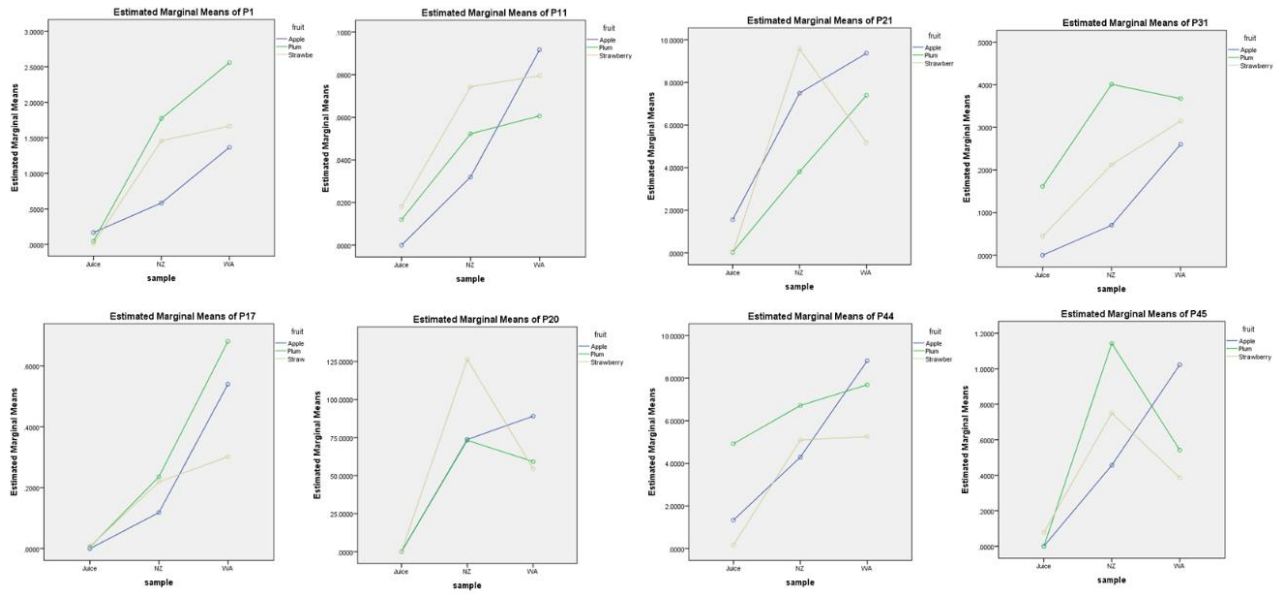
		Juice			ScNZ			ScWA		
Name	**Peak**	**Pl**	**Ap**	**St**	**Pl**	**Ap**	**St**	**Pl**	**Ap**	**St**
1-penten-3-ol	**P12**	✓	✓	✓	x	x	x	x	x	x
3-hydroxy-2-butanone	**P16**	x	x	x	x	x	x	✓	✓	✓
2,4,5-trimethyl-1,3-dioxolane A	**P19**	x	x	x	✓	✓	✓	✓	✓	✓
2,4,5-trimethyl-1,3-dioxolane B	**P22**	x	x	x	x	x	x	✓	✓	✓
2-methylethyl propanoate	**P25**	x	x	x	✓	✓	✓	✓	✓	✓
hexanal	**P30**	✓	✓	✓	✓	x	x	✓	x	x
2-hexenal A	**P36**	✓	✓	✓	x	x	x	x	x	x
2-hexenal B	**P38**	✓	✓	✓	x	x	x	x	x	x
3-methyl pentanol	**P39**	x	x	x	✓	✓	✓	x	✓	✓
Unknown 2	**P40**	x	x	x	✓	✓	✓	✓	✓	✓
2-hexen-1-ol	**P43**	✓	✓	✓	x	x	x	x	x	x
heptanal	**P52**	✓	✓	✓	x	x	x	x	x	x
NoID	**P61**	x	x	x	✓	✓	✓	✓	✓	✓
methyl 2-hydroxy-4-methylpentanoate	**P62**	x	x	x	✓	✓	✓	✓	✓	✓
2-methylthiolan-3-one	**P65**	x	x	x	✓	✓	✓	✓	✓	✓
1-heptanol	**P69**	x	x	x	✓	✓	✓	✓	✓	✓
butyl butanoate	**P75**	✓	✓	✓	x	x	x	x	x	x
2-phenylethanol	**P94**	x	x	x	✓	✓	✓	✓	✓	✓
Ethyl octanoate	**P106**	x	x	x	✓	✓	✓	✓	✓	✓
2-phenethyl acetate	**P115**	x	x	x	✓	✓	✓	✓	✓	✓

TableSC3 Multivariate analysis of volatiles quantified from sterile and inoculated juice (*Saccharomyces cerevisiae* ScNZ and *S. cerevisiae* ScWA, N=6) in plum, apple, strawberry. MANOVA was employed and corrected for multiple comparisons using Tukey HSD to test for the null-hypothesis that standard-mean levels of volatiles are equal and yeast has no effect on volatile composition. $P<0.05$ rejects this null hypothesis and indicates that compound levels were consistently different. The asterisk highlights volatiles different in sterile compared to inoculated juice across all fruit types.

		***P* (Juice-ScNZ)**			***P*(Juice-ScWA)**			***P*(ScNZ-ScWA)**		
Name	**Peak**	**Pl**	**Ap**	**St**	**Pl**	**Ap**	**St**	**Pl**	**Ap**	**St**
1-propanol	**P1**	0.001			<0.001			0.114		
2,3-butanedione	**P2**	0.077			0.362			0.591		
ethyl acetate	**P5**	0.001			0.250			0.026		
2-methyl propanol	**P6**	0.008			0.888			0.009		
1-butanol*	**P9**	<0.001			0.001			0.964		
2-pentanone*	**P11**	<0.001			<0.001			0.009		
Unknown 1	**P13**	0.082			0.305			0.697		
ethyl propanoate*	**P17**	0.024			<0.001			<0.001		
3-methyl butanol*	**P20**	<0.001			<0.001			0.054		
2-methyl butanol*	**P21**	<0.001			<0.001			0.998		
2-methylpropyl acetate	**P27**	0.952			0.596			0.707		
hexanal*	**P30**	<0.001			<0.001			0.985		
ethyl butanoate*	**P31**	0.028			<0.001			0.084		
3-hexenol	**P42**	0.568			0.579			1		
1-hexanol*	**P44**	0.001			<0.001			0.016		
3-methylbutyl acetate*	**P45**	<0.001			<0.001			0.853		
2-methylbutyl acetate*	**P46**	0.013			0.017			0.997		
ethyl hexanoate	**P77**	0.104			<0.001			<0.001		
linalool	**P96**	0.414			0.582			0.947		
octanoic Acid	**P104**	0.069			<0.001			<0.001		
nonanoic acid	**P124**	0.072			0.044			0.954		
beta damascenone*	**P129**	0.023			0.039			0.972		



FigureSC3: Volatiles that significantly decreased after inoculation of sterile plum, apple and strawberry juice with *Saccharomyces cerevisiae* Fly_KR78.3 (ScNZ) and *S. cerevisiae* DBVP6044 (ScWA), N=6, TukeyHSD-corrected MANOVA): butanol (P9), hexanal (P30), 2-methylbutyl acetate (P46), β-damascenone (P129).



FigureSC4: Volatiles that significantly increased after inoculation of sterile plum, apple and strawberry juice with *Saccharomyces cerevisiae* ScNZ and *S. cerevisiae Sc*WA, N=6, TukeyHSD-corrected MANOVA): Propanol (P1), 2-Pentanone (P11), ethyl propanoate (P17), 3-methylbutanol (P20), 2-methylbutanol (P21), hexanol (P44), 3-methylbutyl acetate (P45).

TableSC4: Non-parametric analysis of yeast volatiles in strawberry ferments using Mann-Whitney U statistics comparing *Saccharomyces cerevisiae* ScNZ and *S. cerevisiae Sc*WA (N=6, α=0.05).

Test Statistics[a]

	P1	P5	P6	P9	P17	P20	P21	P25	P27	P31	P44	P45	P46	P69	P77	P80	P94	P106	P115
Mann-Whitney U	18.000	.000	.000	7.000	9.000	.000	5.000	6.000	7.500	6.000	18.000	7.000	10.000	.000	1.000	.000	.000	4.500	13.000
Wilcoxon W	39.000	21.000	21.000	28.000	30.000	21.000	26.000	27.000	28.500	27.000	39.000	28.000	31.000	21.000	22.000	21.000	21.000	25.500	34.000
Z	.000	-2.882	-2.882	-1.761	-1.441	-2.882	-2.082	-1.922	-1.684	-1.925	.000	-1.761	-1.283	-2.882	-2.722	-3.077	-2.882	-2.166	-.802
Asymp. Sig. (2-tailed)	1.000	.004	.004	.078	.150	.004	.037	.055	.092	.054	1.000	.078	.199	.004	.006	.002	.004	.030	.423
Exact Sig. [2*(1-tailed Sig.)]	1.000[b]	.002[b]	.002[b]	.093[b]	.180[b]	.002[b]	.041[b]	.065[b]	.093[b]	.065[b]	1.000[b]	.093[b]	.240[b]	.002[b]	.004[b]	.002[b]	.002[b]	.026[b]	.485[b]

a. Grouping Variable: group

b. Not corrected for ties.

Supplemental D

TableSD1: Summary of the full factorial ANOVA results for each chemical compound against fruit type and yeast genotype. The P-values have been adjusted for multiple comparisons. F-statistics above 5 and P-values below 0.05 are highlighted in red.

Peak	Compound	Fruit		Yeast		Fruit*Yeast	
		F-stat	*P*-value	F-stat	*P*-value	F-stat	*P*-value
P1	1-propanol	11.44	0.001	11.46	0.004	1.67	0.238
P2	2,3-butanedione	20.97	0.000	17.22	0.001	18.06	0.000
P5	ethyl acetate	11.38	0.001	11.17	0.004	8.59	0.002
P6	2-methyl propanol	75.51	0.000	77.98	0.000	83.46	0.000
P9	1-butanol	31.32	0.000	8.88	0.010	2.23	0.152
P11	2-pentanone	3.42	0.063	13.41	0.002	4.79	0.024

P13	Unknown 1	28.81	0.000	1.04	0.345	1.16	0.352
P17	ethyl propanoate	4.04	0.041	41.19	0.000	5.53	0.015
P20	3-methyl butanol	3.33	0.065	7.55	0.016	12.33	0.000
P21	2-methyl butanol	3.94	0.043	0.42	0.537	10.95	0.001
P25	2-methylethyl propanoate	2.55	0.119	0.36	0.555	3.06	0.080
P27	2-methylpropyl acetate	38.47	0.000	4.50	0.060	10.07	0.001
P31	ethyl butanoate	11.58	0.001	6.80	0.022	0.65	0.540
P39	3-methyl pentanol	29.22	0.000	0.39	0.540	5.81	0.012
P40	Unknown 2	38.53	0.000	1.22	0.315	1.22	0.342
P42	3-hexenol	2065.74	0.000	22.78	0.000	6.77	0.007
P44	1-hexanol	9.01	0.002	26.33	0.000	10.64	0.001
P45	3-methylbutyl acetate	3.37	0.064	1.70	0.237	15.95	0.000
P46	2-methylbutyl acetate	7.63	0.004	1.47	0.268	6.55	0.008
P61	Unknown 3	4.62	0.027	4.27	0.064	18.41	0.000
P62	methyl 2-hydroxy-4-methyl pentanoate	1.29	0.323	10.01	0.006	23.41	0.000
P65	2-methylthiolan-3-one	28.93	0.000	3.39	0.097	11.31	0.001
P69	1-heptanol	0.92	0.430	117.82	0.000	2.80	0.097
P77	ethyl hexanoate	12.51	0.000	51.75	0.000	3.97	0.043
P80	hexyl acetate	14.06	0.000	8.33	0.012	26.56	0.000
P94	2-phenylethanol	36.17	0.000	10.89	0.005	22.79	0.000
P96	linalool	129.37	0.000	0.49	0.510	1.03	0.395
P104	octanoic acid	2.20	0.154	47.90	0.000	4.73	0.025
P106	ethyl octanoate	16.81	0.000	95.59	0.000	11.42	0.001
P115	2-phenethyl acetate	26.98	0.000	20.36	0.000	18.78	0.000
P124	nonanoic acid	10.85	0.001	2.33	0.163	12.20	0.000
P129	beta-damascenone	10.58	0.001	4.38	0.063	2.50	0.122

TableSD2: Summary of the PERMANOVA results testing the effects of fruit type and yeast strain on the chemical composition of ferments

Factor	Degrees of Freedom	F-statistic	R^2 value	*P*-value
Fruit	2	6.29	0.189	1×10^{-4}
Yeast	1	6.09	0.091	9×10^{-4}
Fruit*Yeast	2	9.02	0.270	1×10^{-4}
Residuals	30		0.450	
Total	35		1	

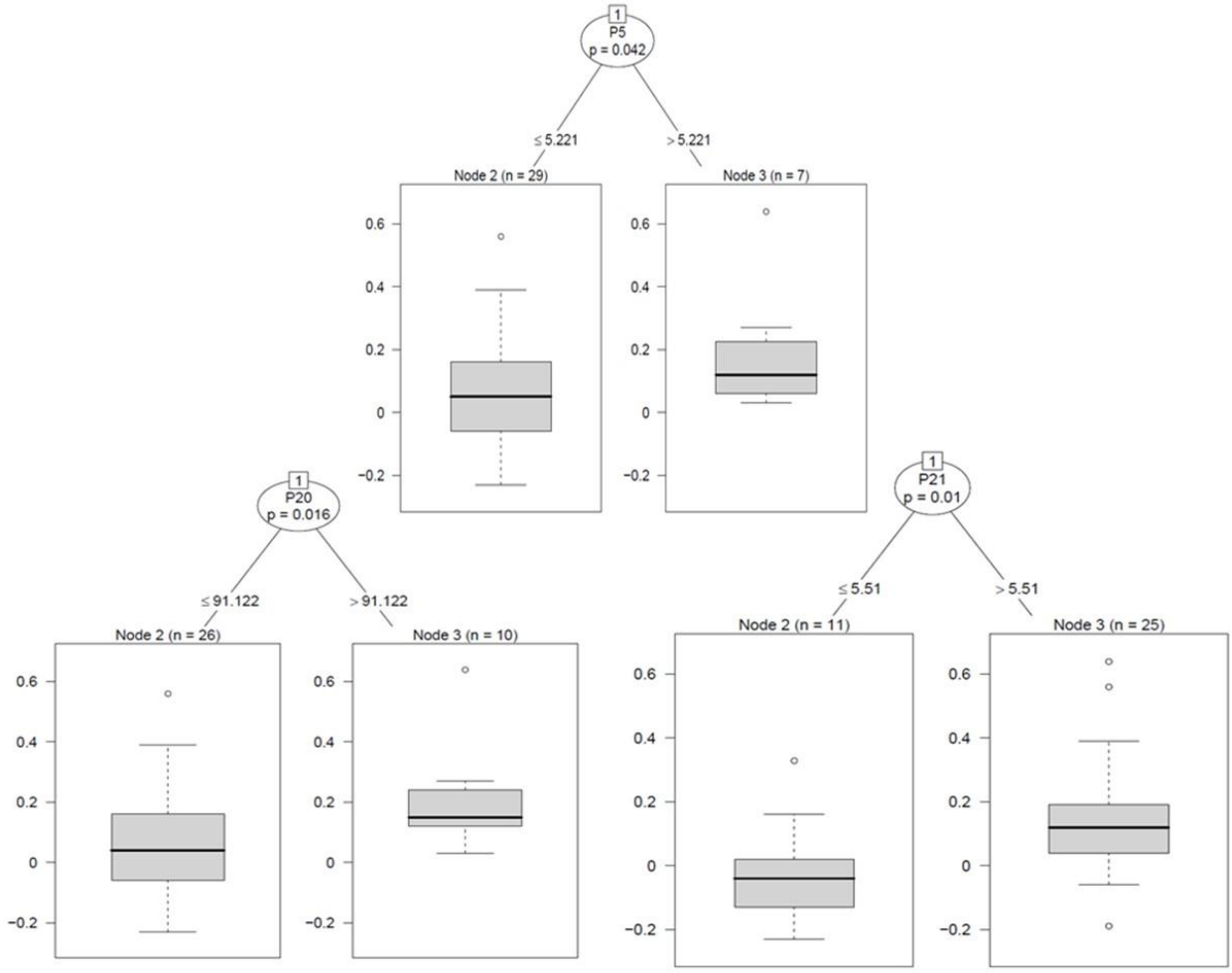
Supplemental E

TableSE1 Summary of test statistics and P-values of correlation tests between each chemical compound and the attraction index for *Drosophila simulans* and *D. melanogaster*. Marginally non-significant correlations are highlighted in red.

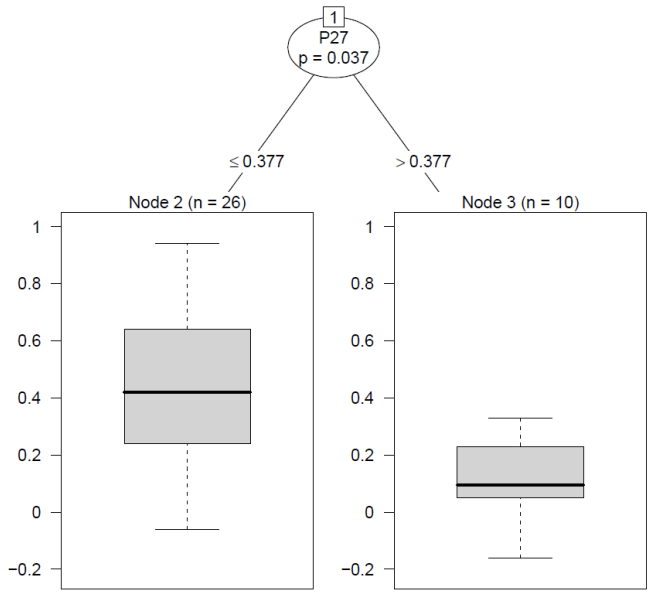
		D. simulans				*D. melanogaster*			
Peak	**Compound**	**t-stats**	**r-value**	**p-value**	**adjusted p-value**	**t-stats**	**r-value**	**p-value**	**adjusted p-value**
P1	1-propanol	-0.329	-0.056	0.744	0.929	-1.754	-0.288	0.088	0.856
P2	2,3-butanedione	1.027	0.174	0.311	0.906	-0.911	-0.154	0.369	0.978
P5	ethyl acetate	2.129	0.343	0.041	0.433	1.267	0.212	0.214	0.856
P6	2-methyl propanol	1.833	0.300	0.076	0.522	1.012	0.171	0.319	0.978
P9	1-butanol	0.536	0.092	0.595	0.929	0.160	0.028	0.873	0.978
P11	2-pentanone	0.210	0.036	0.835	0.93	0.608	0.104	0.547	0.978
P13	Unknown 1	0.629	0.107	0.534	0.929	-0.098	-0.017	0.922	0.978
P17	ethyl propanoate	0.437	0.075	0.665	0.929	-0.553	-0.094	0.584	0.978
P20	3-methyl butanol	2.601	0.407	0.014	0.218	0.417	0.071	0.679	0.978
P21	2-methyl butanol	2.807	0.434	0.008	0.218	0.185	0.032	0.854	0.978
P25	2-methylethyl propanoate	1.517	0.252	0.138	0.554	0.191	0.033	0.850	0.978
P27	2-methylpropyl acetate	-0.331	-0.057	0.742	0.929	-2.192	-0.352	0.035	0.856
P31	ethyl butanoate	-0.310	-0.053	0.758	0.929	-1.373	-0.229	0.179	0.856
P39	3-methyl pentanol	0.765	0.130	0.450	0.929	0.336	0.058	0.739	0.978
P40	Unknown 2	0.505	0.086	0.617	0.929	-1.332	-0.223	0.192	0.856
P42	3-hexenol	-0.316	-0.054	0.754	0.929	-1.450	-0.241	0.156	0.856
P44	1-hexanol	0.277	0.047	0.784	0.929	-0.830	-0.141	0.412	0.978

P45	3-methylbutyl acetate	0.061	0.010	0.952	0.967	-1.290	-0.216	0.206	0.856
P46	2-methylbutyl acetate	1.250	0.210	0.220	0.703	0.046	0.008	0.964	0.978
P61	Unknown 3	1.795	0.294	0.082	0.522	0.905	0.153	0.372	0.978
P62	methyl 2-hydroxy-4-methyl pentanoate	0.561	0.096	0.579	0.929	1.584	0.262	0.122	0.856
P65	2-methylthiolan-3-one	1.619	0.268	0.115	0.554	0.789	0.134	0.435	0.978
P69	1-heptanol	-0.162	-0.028	0.872	0.93	-0.391	-0.067	0.698	0.978
P77	ethyl hexanoate	-0.576	-0.098	0.569	0.929	-0.124	-0.021	0.902	0.978
P80	hexyl acetate	1.382	0.231	0.176	0.626	0.418	0.071	0.679	0.978
P94	2-phenylethanol	0.187	0.032	0.853	0.93	-0.132	-0.023	0.895	0.978
P96	Linalool	-0.432	-0.074	0.669	0.929	0.355	0.061	0.725	0.978
P104	octanoic acid	-0.042	-0.007	0.967	0.967	-0.054	-0.009	0.958	0.978
P106	ethyl octanoate	0.582	0.099	0.564	0.929	-0.398	-0.068	0.693	0.978
P115	2-phenethyl acetate	0.702	0.120	0.487	0.929	0.469	0.080	0.642	0.978
P124	nonanoic acid	1.539	0.255	0.133	0.554	-0.868	-0.147	0.392	0.978
P129	damascenone	0.528	0.090	0.601	0.929	-0.027	-0.005	0.978	0.978

Supplemental F



FigSF1: Conditional inference tree analysis for *Drosophila simulans* attraction (Attraction Index on y-axis) to ethyl acetate (P5), 3-methylbutanol (P20) and 2-methylbutanol (P21) in fruit. This analysis identifies if the data can be split into two groups based on the compounds concentration to create two groups that have statistically different attraction indices (the *P*-value is indicated in the top circle). The two box plots show the distribution of the attraction indices of the two groups created, with the values on the lines connecting the boxes to the top circle indicating the concentration of the compound where the split was made.



FigSF2: Conditional inference tree analysis for *Drosophila melanogaster* attraction (Attraction Index on y-axis) to 2-methylpropyl acetate (P27) in fruit. This analysis identifies if the data can be split into two groups based on the compounds concentration to create two groups that have statistically different attraction indices (the *P*-value is indicated in the top circle). The two box plots show the distribution of the attraction indices of the two groups created, with the values on the lines connecting the boxes to the top circle indicating the concentration of the compound where the split was made.

***Supplemental material 1*: "Are *Drosophila*-preferences for yeasts stable or contextual?"**

by Catrin S. Günther, Sarah J. Knight, Rory Jones and Matthew R. Goddard

Table SMM: Origin of *Saccharomycetaceae* isolates.

Name	Country of origin	Source	Reference
Saccharomyces cerevisiae 'Lalvin' EC-1118	France	Commercial wine yeast	Lallemand inc. Lallemandbrewing.com
Saccharomyces cerevisiae Fly_KR78.3	New Zealand	*Drosophila simulans*	Buser et al 2014. Ecology Letters 17: 157-64
Saccharomyces cerevisiae DBVP6044	West Africa	Bili Wine	Liti et al. 2009. Nature 458: 337-41
Saccharomyces uvarum SBJ1d	New Zealand	Sauvignon Blanc ferment	Goddard culture collection
Hanseniaspora uvarum 11-382	USA	*Drosophila suzukii*	Phaff Yeast culture collection, UC-Davis
Hanseniaspora uvarum HB-62	New Zealand	Chardonnay fruit	Gayevskiy et al. 2012. ISME Journal 6:1281-90
Hanseniaspora occidentalis WI-82	New Zealand	Syrah fruit	
Pichia kluyverii JT3.71	New Zealand	Chardonnay juice	Anfang et al. 2009. Australian Journal of Grape and Wine Research 15:1-8
Pichia pijperi	New Zealand	Pinot noir ferment	Goddard culture collection
Candida apicola X120705S2.1	New Zealand	Beehive	Anfang et al. 2009. Australian Journal of Grape and Wine Research 15:1-8
Candida zemplininia FA2.12	New Zealand	Chardonnay ferment	

Supplemental A

Data were analysed using IBM SPSS Statistics Version 21

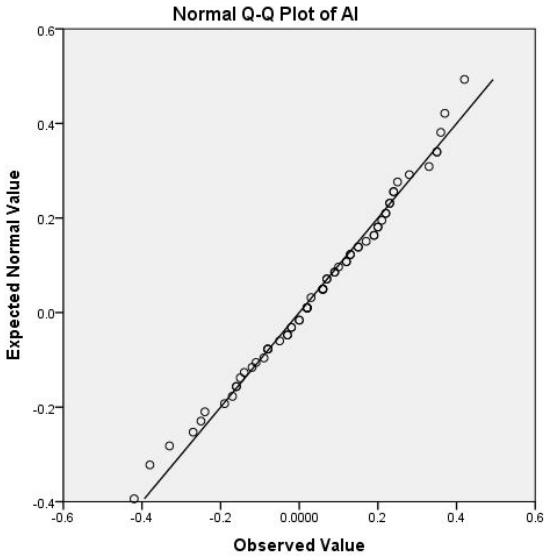


Figure SA1: Q-Q plots indicating roughly normal distribution of combined attraction Indices (AI, N=66) testing *Drosophila simulans* (ancestral line/F0, 3 independent F11 populations) preference for *Saccharomyces cerevisiae*_ ScNZ against *S. cerevisiae* ScWA in two-way choice tests.

TableSA1: Levene's Test of Equality of Error Variances. Tests the null hypothesis of homogeneity of variances in Attraction indices (AI) as the dependent variable across *Drosophila simulans* populations.

F	df1	df2	Sig.
.494	3	62	.687

TableSA2: Paired Samples Test. Testing the null hypothesis that there is no difference in *Drosophila simulans* attraction (AI as dependent variable) for *Saccharomyces cerevisae* ScNZ between the ancestral line (DsF0) and evolved (DsNZF11, DsWAF11) or control/ unevolved offspring population (DsCF11).

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	DsF0 - DsCF11	.05500	.30480	.08799	-.13866	.24866	.625	11	.545
Pair 2	DsF0 - DsNZF11	.07417	.27550	.07953	-.10088	.24921	.933	11	.371
Pair 3	DsF0 - DsWAF11	.22833	.30522	.08811	.03440	.42226	2.591	11	.025

TableSA3: Tukey HSD corrected Multiple Comparisons Testing the null hypothesis that there is no difference in *Drosophila simulans* attraction (AI as dependent variable) for *Saccharomyces cerevisae*_ScNZ between each fly population. DsF0: ancestral line; DsNZF11: evolved to select *S. cerevisiae*_ScNZ; DsWAF11: evolved to select *S. cerevisiae*_ScWA; DsCF11 control/ unevolved offspring

Population (I)	(J) Population	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
DsF0	DsCF11	.0161	.06528	.995	-.1562	.1884
	DsNZF11	.0494	.06528	.873	-.1229	.2218
	DsWAF11	.1989*	.06528	.017	.0266	.3712
DsF11	DsF0	-.0161	.06528	.995	-.1884	.1562
	DsNZF11	.0333	.05838	.940	-.1208	.1875
	DsWAF11	.1828*	.05838	.014	.0286	.3369
DsNZF11	DsF0	-.0494	.06528	.873	-.2218	.1229
	DsCF11	-.0333	.05838	.940	-.1875	.1208
	DsWAF11	.1494	.05838	.061	-.0047	.3036
DsWAF11	DsF0	-.1989*	.06528	.017	-.3712	-.0266
	DsCF11	-.1828*	.05838	.014	-.3369	-.0286
	DsNZF11	-.1494	.05838	.061	-.3036	.0047

Based on observed means.

The error term is Mean Square(Error) = .031.

*. The mean difference is significant at the .05 level.

~~Supplemental B~~

TableSB1: Identified peaks in gas chromatograms of different runs and tentative compound identification based on comparison of its retention time (RT) and mass spectrum with the NIST05 database.

Peak	Name	RT	Peak	Name	RT
P1	propanol	1.829	P66	4-butoxy-2-butanone	11.388
P2	2,3-butanedione	1.919	P67	3-(methylthio)propanol	11.489
P3	1-methylpropyl formate	2.06	P68	3-methylbutyl propanoate	11.743
P4	2-methyl furan	2.103	P69	1-heptanol	11.83
P5	ethyl acetate	2.133	P70	8,8-dimethoxy-2-octanyl 2-formyl-4,6-dimethoxybenzoate	12.054
P6	2-methyl propanol	2.292	P71	6-methyl-5-hepten-2-one	12.176
P7	3-methyl butanal	2.453	P72	1-octen-3-ol (mushroom alcohol)	12.71
P8	2-methyl butanal	2.566	P73	7-octen-2-ol	12.273
P9	1-butanol	2.637	P74	6-methyl-5-hepten-2-ol	12.848
P10	1-penten-3-one	2.794	P75	butyl butanoate	12.937
P11	2-pentanone	2.817	P76	hexyl butanoate	12.942
P12	1-penten-3-ol	2.851	P77	ethyl hexanoate	13.107
P13	2,3-pentanedione	2.896	P78	3-hexenyl acetate	13.342
P14	Pentanal	2.919	P79	3-eicosyne	13.503
P15	ethylmethyl carbonate	3.042	P80	hexyl acetate	13.747
P16	3-hydroxy-2-butanone	3.107	P81	benzeneacetaldehyde	14.084
P17	ethyl propanoate	3.256	P82	2-ethylhexanol	14.681
P18	methyl butanoate	3.437	P83	ethyl 2-hexenoate	14.957
P19	2,4,5-trimethyl-1,3-dioxolane Isomer 1	3.581	P84	1-methylethyl hexanoate	15.02
P20	3-methyl butanol	3.96	P85	butyl 2-methylbutanoate	15.212
P21	2-methyl butanol	3.99	P86	2,5-dimethyl-4-methoxy-3(2H)-furanone (strawberry furanone methyl ether)	15.313
Peak	Name	RT	Peak	Name	RT

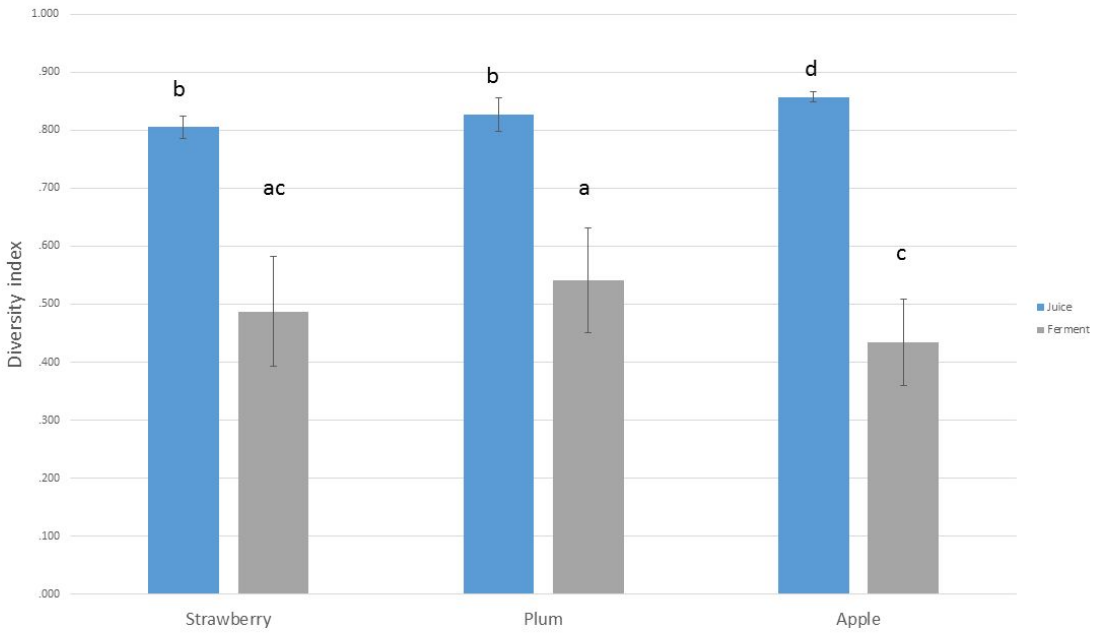
P22	2,4,5-trimethyl-1,3-dioxolane Isomer 2	4.109	P87	cis-linalool oxide	16.485
P23	methyl 2-butenoate	4.156	P88	octanol	16.631
P24	1-methylpropyl acetate	4.22	P89	2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol)	16.749
P25	2-methylethyl propanoate	4.274	P90	2-methylpropyl propanoate	17.076
P26	1-pentanol	4.483	P91	trans linalool oxide	17.174
P27	2-methylpropyl acetate	4.573	P92	3-hexenyl butanoate	17.267
P28	2,3-butanediol, [R-(R*,R*)]-	4.654	P93	heptanoic acid	17.72
P29	methyl 2-methylbutanoate	4.714	P94	2-phenylethyl Alcohol	17.76
P30	hexanal	5.038	P95	nonanal	17.803
P31	ethyl butanoate	5.173	P96	linalool	17.903
P32	2-methylpropanoic acid	5.265	P97	2-butoxyethyl acetate	18.207
P33	butyl acetate	5.615	P98	3-hydroxybutyl butanoate	18.94
P34	methyl pentanoate	5.926	P99	2-ethylhexanoic acid	19.56
P35	2,3- heptanedione (acetyl valeryl)	6.188	P100	2-phenylmethyl acetate	20.13
P36	2-hexenal Isomer A	6.293	P101	epoxylinalol	21.159
P37	4-methyl pentanol	6.462	P102	1-nonanol	21.363
P38	2-hexenal Isomer B	6.714	P103	p-menth-1-en-8-ol	21.846
P39	3-methyl pentanol	6.719	P104	octanoic Acid	21.953
P40	2-methyl-3-hexanol	6.721	P105	hexyl butanoate	22.329
P41	3-ethoxy propanol	6.784	P106	ethyl octanoate	22.561
P42	3-hexenol	7.022	P107	decanal	22.658
P43	2-hexen-1-ol	7.448	P108	2,3-dihydro-1,1,5,6-tetramethyl-1H-indene	23.133
P44	1-hexanol	7.627	P109	octyl acetate	23.181
P45	3-methylbutyl acetate	7.784	P110	2-propylheptanol	23.268
P46	2-methylbutyl acetate	7.892	P111	5-butyldihydro-2(3H)-Furanone (cis-oak lactone)	23.67

Peak	Name	RT	Peak	Name	RT
P47	3-(methylthio)propanal	7.943	P112	cis-geraniol	23.73
P48	2-methylbutanoic acid	7.967	P113	beta-citronellol	23.804
P49	2-heptanone	8.087	P114	2,7-dimethyl 1-octanol (dehydrocitronellol)	24.116
P50	3-methyl 3-hexanol	8.122	P115	2-phenethyl acetate	24.266
P51	methyl 4-methylpentanoate	8.324	P116	2-methylhexyl butanoate	24.327
P52	heptanal	8.467	P117	transgeraniol	24.792
P53	butyl propanoate	9.075	P118	2-(2-butenyl)-1,3,5-trimethyl-benzene	24.807
P54	2-methylbutenoic acid	9.101	P119	1,3-bis(1,1-dimethylethyl) benzene	24.987
P55	pentyl acetate	9.282	P120	2-ethylmethyl hexanoate	25.103
P56	2-pentenyl acetate	9.373	P121	(1,4-dimethylpent-2-enyl)benzene	25.33
P57	pentanoic acid	9.547	P122	1-decanol	25.626
P58	3-methyl-2-butenyl acetate	9.55	P123	(2Z)-2-methyl-5-[(1S,2R,4R)-2-methyl-3-methylidenebicyclo[2.2.1]hept-2-yl]pent-2-en-1-ol (beta-Santalol)	25.893
P59	methyl hexanoate	9.703	P124	nonanoic acid	26.249
P60	5-methyl heptanone	9.86	P125	2-butyloctanol	27.115
P61	No ID	10.146	P126	3-hydroxybutyl butanoate	27.287
P62	methyl 2-hydroxy-4-methyl pentanoate	10.31	P127	methyl decanoate	27.413
P63	2-methylpropyl butanoate	10.755	P128	2-methyldecanol	27.578
P64	2-methylbutyl propanoate	11.075	P129	beta damascenone	28.952
P65	dihydro-2-methyl-3(2H)-thiophenone (blackberry thiophenone)	11.256			

Supplemental C

Table SC1: Number of different ~~volatile compounds~~ peaks, detected in the headspace of sterile and yeast- inoculated juice

Fruit type	sample	N volatiles
Strawberry	Yeast ScNZ	46
	Yeast ScWA	44
	juice	60
Plum	Yeast ScNZ	41
	Yeast ScWA	44
	juice	49
Apple	Yeast ScNZ	35
	Yeast ScWA	41
	juice	51



FigSC1: Chemical diversity of headspace volatiles from inoculated (blue) and sterile (grey) juice. The error bars represent the standard deviation of the mean diversity index (Simpson's diversity) reflecting counts and concentrations of chemical compounds. Kruskal Wallis-H test (X^2=40.4; p<0.0001) indicates a significantly different diversity distribution between samples. This has been confirmed for pairwise comparison using the Mann-Whitney U test, except for samples highlighted with the same letters which are not significantly different from each other in their median chemical diversity.

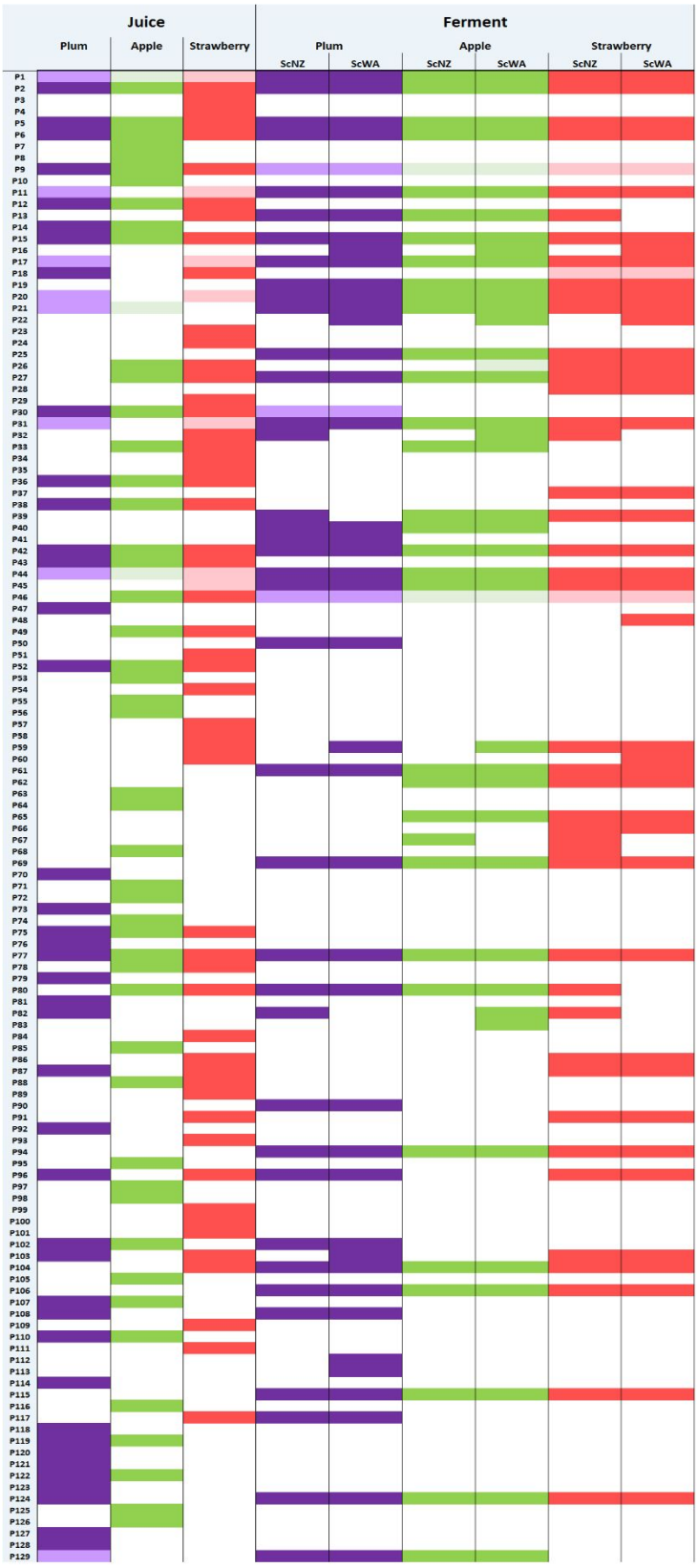


Figure SC2: Volatile profiles of sterile fruit juice and after inoculation with *Saccharomyces cerevisiae* strain ScNZ and ScWA, respectively. Increased colour transparency indicates that compound levels were significantly (P<0.05) lower when compared to the sterile or inoculated sample of the corresponding fruit (TukeyHSD-corrected Manova, see TableSD1 and SD2).

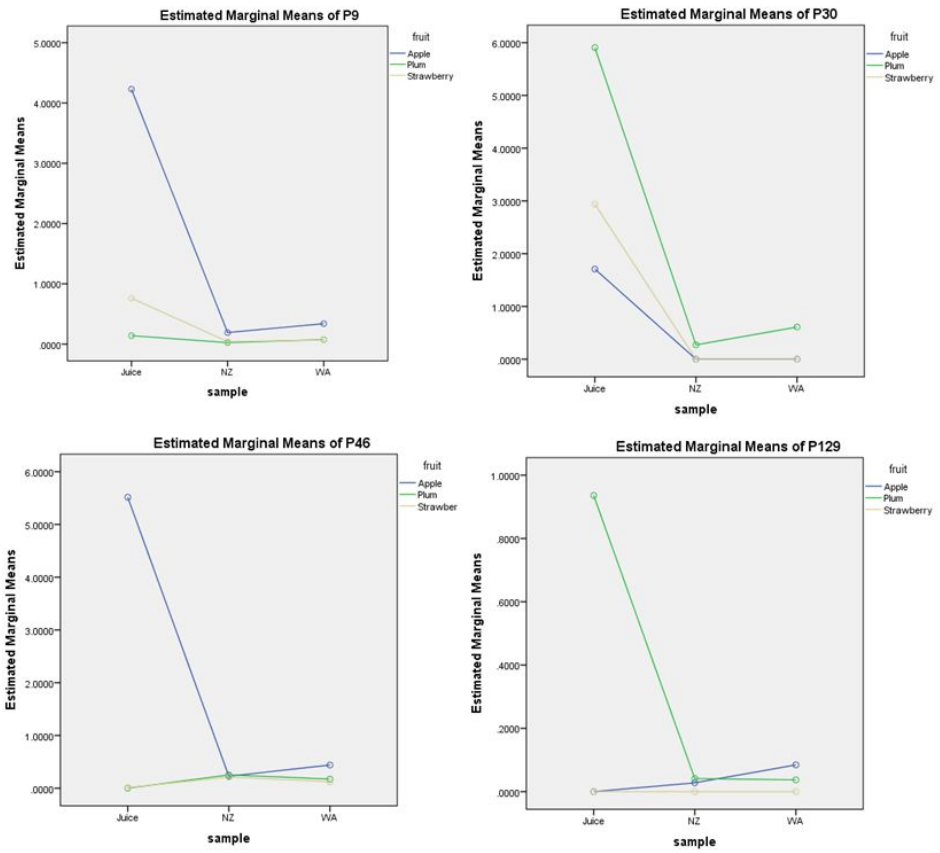
TableSC2: Fruit-specific and yeast specific compounds detected in sterile plum (Pl), apple (Ap) and strawberry (St) juice and after inoculation with *Saccharomyces cerevisiae* ScNZ and *S. cerevisiae* ScWA, respectively. ✓ indicates that the compound is present in a sample and x that is was not detected.

		Juice			ScNZ			ScWA		
Name	**Peak**	**Pl**	**Ap**	**St**	**Pl**	**Ap**	**St**	**Pl**	**Ap**	**St**
1-penten-3-ol	**P12**	✓	✓	✓	x	x	x	x	x	x
3-hydroxy-2-butanone	**P16**	x	x	x	x	x	x	✓	✓	✓
2,4,5-trimethyl-1,3-dioxolane A	**P19**	x	x	x	✓	✓	✓	✓	✓	✓
2,4,5-trimethyl-1,3-dioxolane B	**P22**	x	x	x	x	x	x	✓	✓	✓
2-methylethyl propanoate	**P25**	x	x	x	✓	✓	✓	✓	✓	✓
hexanal	**P30**	✓	✓	✓	✓	x	x	✓	x	x
2-hexenal A	**P36**	✓	✓	✓	x	x	x	x	x	x
2-hexenal B	**P38**	✓	✓	✓	x	x	x	x	x	x
3-methyl pentanol	**P39**	x	x	x	✓	✓	✓	x	✓	✓
Unknown 2 ~~2-methyl-3-hexanol~~	**P40**	x	x	x	✓	✓	✓	✓	✓	✓
2-hexen-1-ol	**P43**	✓	✓	✓	x	x	x	x	x	x
heptanal	**P52**	✓	✓	✓	x	x	x	x	x	x
NoID	**P61**	x	x	x	✓	✓	✓	✓	✓	✓
methyl 2-hydroxy-4-methylpentanoate	**P62**	x	x	x	✓	✓	✓	✓	✓	✓
2-methylthiolan-3-one	**P65**	x	x	x	✓	✓	✓	✓	✓	✓
1-heptanol	**P69**	x	x	x	✓	✓	✓	✓	✓	✓
butyl butanoate	**P75**	✓	✓	✓	x	x	x	x	x	x
2-phenylethanol	**P94**	x	x	x	✓	✓	✓	✓	✓	✓
Ethyl octanoate	**P106**	x	x	x	✓	✓	✓	✓	✓	✓
2-phenethyl acetate	**P115**	x	x	x	✓	✓	✓	✓	✓	✓

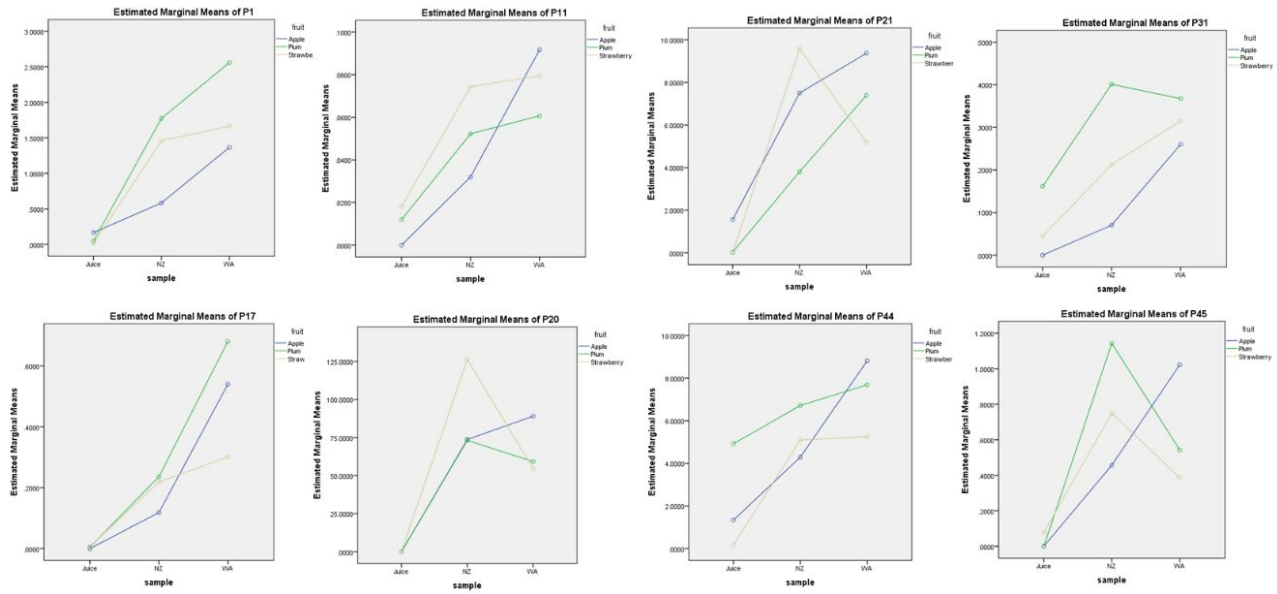
***P*(Juice-ScNZ)** ***P*(Juice-ScWA)** ***P*(ScNZ-ScWA)**

Name	Peak	Pl	Ap	St	Pl	Ap	St	Pl	Ap	St
1-propanol	**P1**		0.001			<0.001			0.114	
2,3-butanedione	**P2**		0.077			0.362			0.591	
ethyl acetate	**P5**		0.001			0.250			0.026	
2-methyl propanol	**P6**		0.008			0.888			0.009	
1-butanol*	**P9**		<0.001			0.001			0.964	
2-pentanone*	**P11**		<0.001			<0.001			0.009	
~~2,3-pentanedione~~Unknown 1	**P13**		0.082			0.305			0.697	
~~ethylmethyl carbonate~~	~~**P15**~~		~~0.332~~			~~0.424~~			~~0.981~~	
ethyl propanoate*	**P17**		0.024			<0.001			<0.001	
3-methyl butanol*	**P20**		<0.001			<0.001			0.054	
2-methyl butanol*	**P21**		<0.001			<0.001			0.998	
2-methylpropyl acetate	**P27**		0.952			0.596			0.707	
hexanal*	**P30**		<0.001			<0.001			0.985	
ethyl butanoate*	**P31**		0.028			<0.001			0.084	
3-hexenol	**P42**		0.568			0.579			1	
1-hexanol*	**P44**		0.001			<0.001			0.016	
3-methylbutyl acetate*	**P45**		<0.001			<0.001			0.853	
2-methylbutyl acetate*	**P46**		0.013			0.017			0.997	
ethyl hexanoate	**P77**		0.104			<0.001			<0.001	
linalool	**P96**		0.414			0.582			0.947	
octanoic Acid	**P104**		0.069			<0.001			<0.001	
nonanoic acid	**P124**		0.072			0.044			0.954	
beta damascenone*	**P129**		0.023			0.039			0.972	

TableSC3 Multivariate analysis of volatiles quantified from sterile and inoculated juice (*Saccharomyces cerevisiae* ScNZ and *S. cerevisiae* ScWA, N=6) in plum, apple, strawberry. MANOVA was employed and corrected for multiple comparisons using Tukey HSD to test for the null-hypothesis that standard-mean levels of volatiles are equal and yeast has no effect on volatile composition. $P<0.05$ rejects this null hypothesis and indicates that compound levels were consistently different. The asterisk highlights volatiles different in sterile compared to inoculated juice across all fruit types.



FigureSC3: Volatiles that significantly decreased after inoculation of sterile plum, apple and strawberry juice with *Saccharomyces cerevisiae* Fly_KR78.3 (ScNZ) and *S. cerevisiae* DBVP6044 (ScWA), N=6, TukeyHSD-corrected MANOVA): butanol (P9), hexanal (P30), 2-methylbutyl acetate (P46), β-damascenone (P129).



FigureSC4: Volatiles that significantly increased after inoculation of sterile plum, apple and strawberry juice with *Saccharomyces cerevisiae* ScNZ and *S. cerevisiae Sc*WA, N=6, TukeyHSD-corrected MANOVA): Propanol (P1), 2-Pentanone (P11), ethyl propanoate (P17), 3-methylbutanol (P20), 2-methylbutanol (P21), hexanol (P44), 3-methylbutyl acetate (P45).

TableSC4: Non-parametric analysis of yeast volatiles in strawberry ferments using Mann-Whitney U statistics comparing *Saccharomyces cerevisiae* ScNZ and *S. cerevisiae Sc*WA (N=6, α=0.05).

Test Statistics[a]

	P1	P5	P6	P9	P17	P20	P21	P25	P27	P31	P44	P45	P46	P69	P77	P80	P94	P106	P115
Mann-Whitney U	18.000	.000	.000	7.000	9.000	.000	5.000	6.000	7.500	6.000	18.000	7.000	10.000	.000	1.000	.000	.000	4.500	13.000
Wilcoxon W	39.000	21.000	21.000	28.000	30.000	21.000	26.000	27.000	28.500	27.000	39.000	28.000	31.000	21.000	22.000	21.000	21.000	25.500	34.000
Z	.000	-2.882	-2.882	-1.761	-1.441	-2.882	-2.082	-1.922	-1.684	-1.925	.000	-1.761	-1.283	-2.882	-2.722	-3.077	-2.882	-2.166	-.802
Asymp. Sig. (2-tailed)	1.000	.004	.004	.078	.150	.004	.037	.055	.092	.054	1.000	.078	.199	.004	.006	.002	.004	.030	.423
Exact Sig. [2*(1-tailed Sig.)]	1.000[b]	.002[b]	.002[b]	.093[b]	.180[b]	.002[b]	.041[b]	.065[b]	.093[b]	.065[b]	1.000[b]	.093[b]	.240[b]	.002[b]	.004[b]	.002[b]	.002[b]	.026[b]	.485[b]

a. Grouping Variable: group

b. Not corrected for ties.

Supplemental D

TableSD1: Summary of the full factorial ANOVA results for each chemical compound against fruit type and yeast genotype. The P-values have been adjusted for multiple comparisons. F-statistics above 5 and P-values below 0.05 are highlighted in red.

Peak	Compound	Fruit		Yeast		Fruit*Yeast	
		F-stat	*P*-value	F-stat	*P*-value	F-stat	*P*-value
P1	1-propanol	11.44	0.001	11.46	0.004	1.67	0.238
P2	2,3-butanedione	20.97	0.000	17.22	0.001	18.06	0.000
P5	ethyl acetate	11.38	0.001	11.17	0.004	8.59	0.002
P6	2-methyl propanol	75.51	0.000	77.98	0.000	83.46	0.000
P9	1-butanol	31.32	0.000	8.88	0.010	2.23	0.152
P11	2-pentanone	3.42	0.063	13.41	0.002	4.79	0.024

P13	~~2,3-~~ ~~pentanedione~~Unknown 1	28.81	0.000	1.04	0.345	1.16	0.352
P17	ethyl propanoate	4.04	0.041	41.19	0.000	5.53	0.015
P20	3-methyl butanol	3.33	0.065	7.55	0.016	12.33	0.000
P21	2-methyl butanol	3.94	0.043	0.42	0.537	10.95	0.001
P25	2-methylethyl propanoate	2.55	0.119	0.36	0.555	3.06	0.080
P27	2-methylpropyl acetate	38.47	0.000	4.50	0.060	10.07	0.001
P31	ethyl butanoate	11.58	0.001	6.80	0.022	0.65	0.540
P39	3-methyl pentanol	29.22	0.000	0.39	0.540	5.81	0.012
P40	Unknown 2~~2-methyl 3-hexanol~~	38.53	0.000	1.22	0.315	1.22	0.342
P42	3-hexenol	2065.74	0.000	22.78	0.000	6.77	0.007
P44	1-hexanol	9.01	0.002	26.33	0.000	10.64	0.001
P45	3-methylbutyl acetate	3.37	0.064	1.70	0.237	15.95	0.000
P46	2-methylbutyl acetate	7.63	0.004	1.47	0.268	6.55	0.008
P61	~~NoID~~ Unknown 3	4.62	0.027	4.27	0.064	18.41	0.000
P62	methyl 2-hydroxy-4-methyl pentanoate	1.29	0.323	10.01	0.006	23.41	0.000
P65	2-methylthiolan-3-one	28.93	0.000	3.39	0.097	11.31	0.001
P69	1-heptanol	0.92	0.430	117.82	0.000	2.80	0.097
P77	ethyl hexanoate	12.51	0.000	51.75	0.000	3.97	0.043
P80	hexyl acetate	14.06	0.000	8.33	0.012	26.56	0.000
P94	2-phenylethanol	36.17	0.000	10.89	0.005	22.79	0.000
P96	linalool	129.37	0.000	0.49	0.510	1.03	0.395
P104	octanoic acid	2.20	0.154	47.90	0.000	4.73	0.025
P106	ethyl octanoate	16.81	0.000	95.59	0.000	11.42	0.001
P115	2-phenethyl acetate	26.98	0.000	20.36	0.000	18.78	0.000
P124	nonanoic acid	10.85	0.001	2.33	0.163	12.20	0.000
P129	beta-damascenone	10.58	0.001	4.38	0.063	2.50	0.122

TableSD2: Summary of the PERMANOVA results testing the effects of fruit type and yeast strain on the chemical composition of ferments

Factor	Degrees of Freedom	F-statistic	R^2 value	*P*-value
Fruit	2	6.29	0.189	1×10^{-4}
Yeast	1	6.09	0.091	9×10^{-4}
Fruit*Yeast	2	9.02	0.270	1×10^{-4}
Residuals	30		0.450	
Total	35		1	

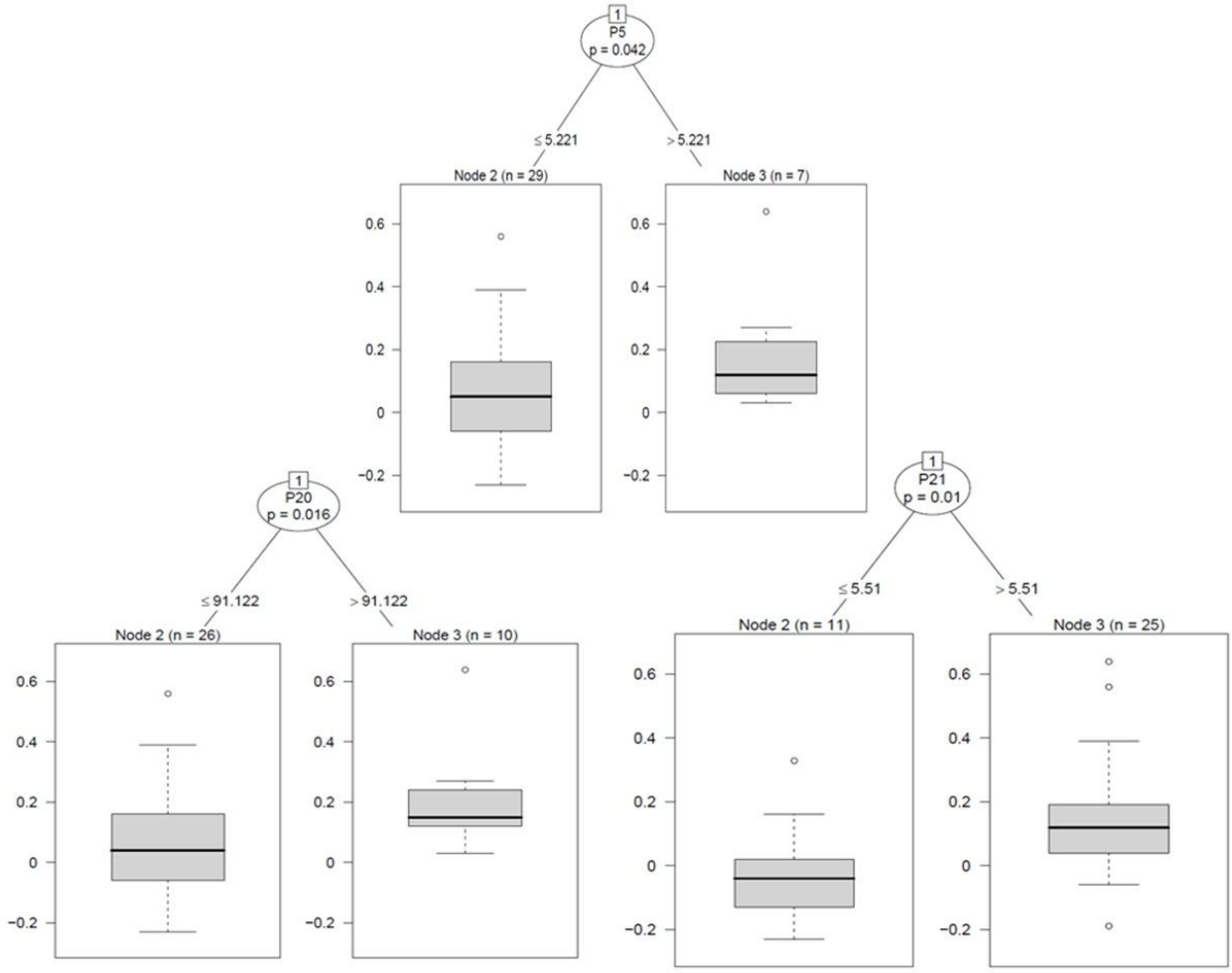
Supplemental E

TableSE1 Summary of test statistics and P-values of correlation tests between each chemical compound and the attraction index for *Drosophila simulans* and *D. melanogaster*. Marginally non-significant correlations are highlighted in red.

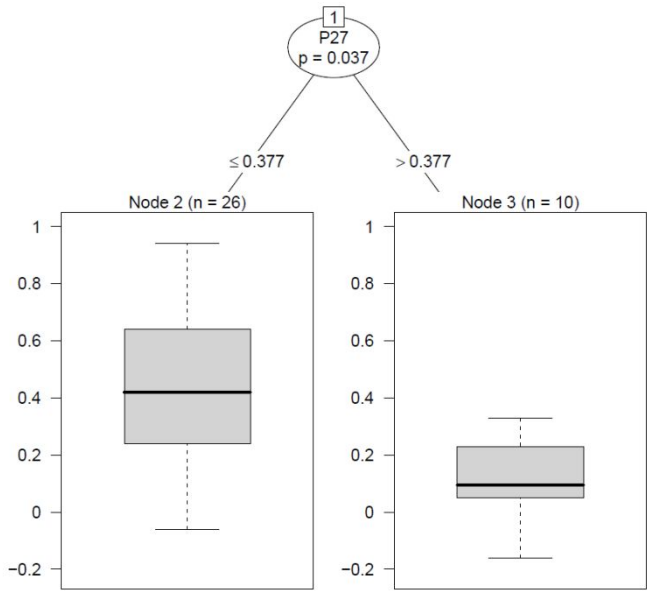
		D. simulans				*D. melanogaster*			
Peak	**Compound**	**t-stats**	**r-value**	**p-value**	**adjusted p-value**	**t-stats**	**r-value**	**p-value**	**adjusted p-value**
P1	1-propanol	-0.329	-0.056	0.744	0.929	-1.754	-0.288	0.088	0.856
P2	2,3-butanedione	1.027	0.174	0.311	0.906	-0.911	-0.154	0.369	0.978
P5	ethyl acetate	2.129	0.343	0.041	0.433	1.267	0.212	0.214	0.856
P6	2-methyl propanol	1.833	0.300	0.076	0.522	1.012	0.171	0.319	0.978
P9	1-butanol	0.536	0.092	0.595	0.929	0.160	0.028	0.873	0.978
P11	2-pentanone	0.210	0.036	0.835	0.93	0.608	0.104	0.547	0.978
P13	~~2,3-pentanedione~~Unknown 1	0.629	0.107	0.534	0.929	-0.098	-0.017	0.922	0.978
P17	ethyl propanoate	0.437	0.075	0.665	0.929	-0.553	-0.094	0.584	0.978
P20	3-methyl butanol	2.601	0.407	0.014	0.218	0.417	0.071	0.679	0.978
P21	2-methyl butanol	2.807	0.434	0.008	0.218	0.185	0.032	0.854	0.978
P25	2-methylethyl propanoate	1.517	0.252	0.138	0.554	0.191	0.033	0.850	0.978
P27	2-methylpropyl acetate	-0.331	-0.057	0.742	0.929	-2.192	-0.352	0.035	0.856
P31	ethyl butanoate	-0.310	-0.053	0.758	0.929	-1.373	-0.229	0.179	0.856
P39	3-methyl pentanol	0.765	0.130	0.450	0.929	0.336	0.058	0.739	0.978
P40	~~2-methyl 3-hexanol~~Unknown 2	0.505	0.086	0.617	0.929	-1.332	-0.223	0.192	0.856

P42	3-hexenol	-0.316	-0.054	0.754	0.929	-1.450	-0.241	0.156	0.856
P44	1-hexanol	0.277	0.047	0.784	0.929	-0.830	-0.141	0.412	0.978
P45	3-methylbutyl acetate	0.061	0.010	0.952	0.967	-1.290	-0.216	0.206	0.856
P46	2-methylbutyl acetate	1.250	0.210	0.220	0.703	0.046	0.008	0.964	0.978
P61	~~NoID~~ Unknown 3	1.795	0.294	0.082	0.522	0.905	0.153	0.372	0.978
P62	methyl 2-hydroxy-4-methyl pentanoate	0.561	0.096	0.579	0.929	1.584	0.262	0.122	0.856
P65	2-methylthiolan-3-one	1.619	0.268	0.115	0.554	0.789	0.134	0.435	0.978
P69	1-heptanol	-0.162	-0.028	0.872	0.93	-0.391	-0.067	0.698	0.978
P77	ethyl hexanoate	-0.576	-0.098	0.569	0.929	-0.124	-0.021	0.902	0.978
P80	hexyl acetate	1.382	0.231	0.176	0.626	0.418	0.071	0.679	0.978
P94	2-phenylethanol	0.187	0.032	0.853	0.93	-0.132	-0.023	0.895	0.978
P96	Linalool	-0.432	-0.074	0.669	0.929	0.355	0.061	0.725	0.978
P104	octanoic acid	-0.042	-0.007	0.967	0.967	-0.054	-0.009	0.958	0.978
P106	ethyl octanoate	0.582	0.099	0.564	0.929	-0.398	-0.068	0.693	0.978
P115	2-phenethyl acetate	0.702	0.120	0.487	0.929	0.469	0.080	0.642	0.978
P124	nonanoic acid	1.539	0.255	0.133	0.554	-0.868	-0.147	0.392	0.978
P129	damascenone	0.528	0.090	0.601	0.929	-0.027	-0.005	0.978	0.978

Supplemental F



FigSF1: Conditional inference tree analysis for *Drosophila simulans* attraction (Attraction Index on y-axis) to ethyl acetate (P5), 3-methylbutanol (P20) and 2-methylbutanol (P21) in fruit. This analysis identifies if the data can be split into two groups based on the compounds concentration to create two groups that have statistically different attraction indices (the *P*-value is indicated in the top circle). The two box plots show the distribution of the attraction indices of the two groups created, with the values on the lines connecting the boxes to the top circle indicating the concentration of the compound where the split was made.



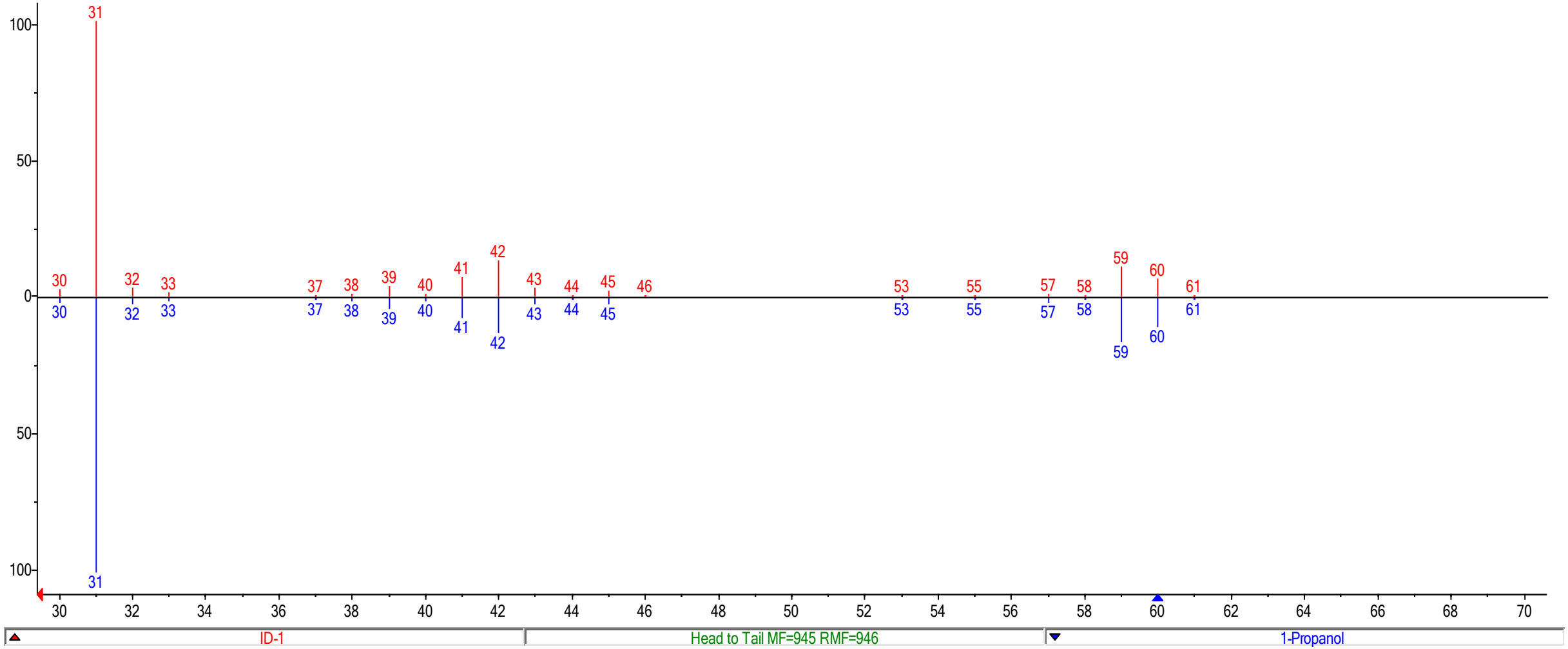
FigSF2: Conditional inference tree analysis for *Drosophila melanogaster* attraction (Attraction Index on y-axis) to 2-methylpropyl acetate (P27) in fruit. This analysis identifies if the data can be split into two groups based on the compounds concentration to create two groups that have statistically different attraction indices (the *P*-value is indicated in the top circle). The two box plots show the distribution of the attraction indices of the two groups created, with the values on the lines connecting the boxes to the top circle indicating the concentration of the compound where the split was made.

Supplemental material B: Are *Drosophila*-preferences for yeasts stable or contextual?"
by Catrin S. Günther, Sarah J. Knight, Rory Jones and Matthew R. Goddard

Mass-spectral (MS) data matches against NIST –mainlibrary (2017) using MS Search 2.2 for tentative compound identification (ID) of volatiles listed in Table 1 using strawberry juice inoculated with *Saccharomyces cerevisiae* as reference.

The given retention time (RT) refers to peaks identified using gas chromatography as described in Materials and Methods. Respective MS- spectra are displayed in red (head-position) and the spectral match of suggested compound-IDs (Table 1) are displayed in blue (tail-position). Compounds are classified as 'Unknown' (displayed in black) when spectra could not be assigned with confidence.

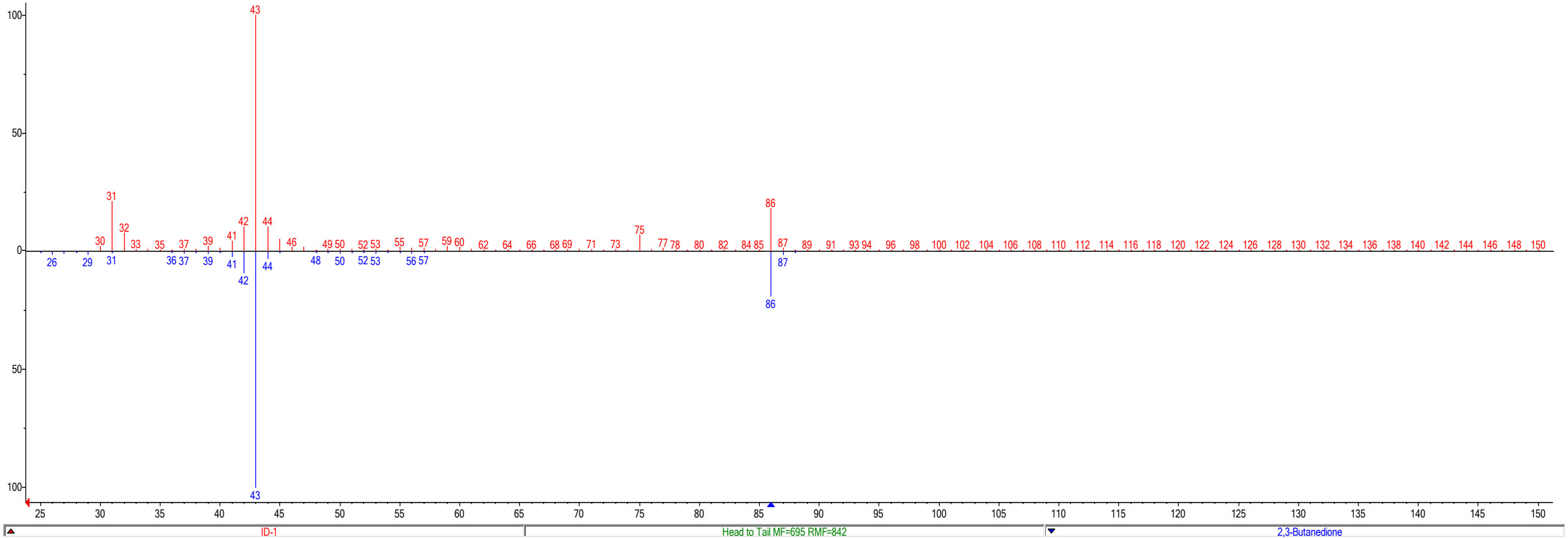
Peak # 1;
RT: 1.829 min
Suggested ID: 1-propanol



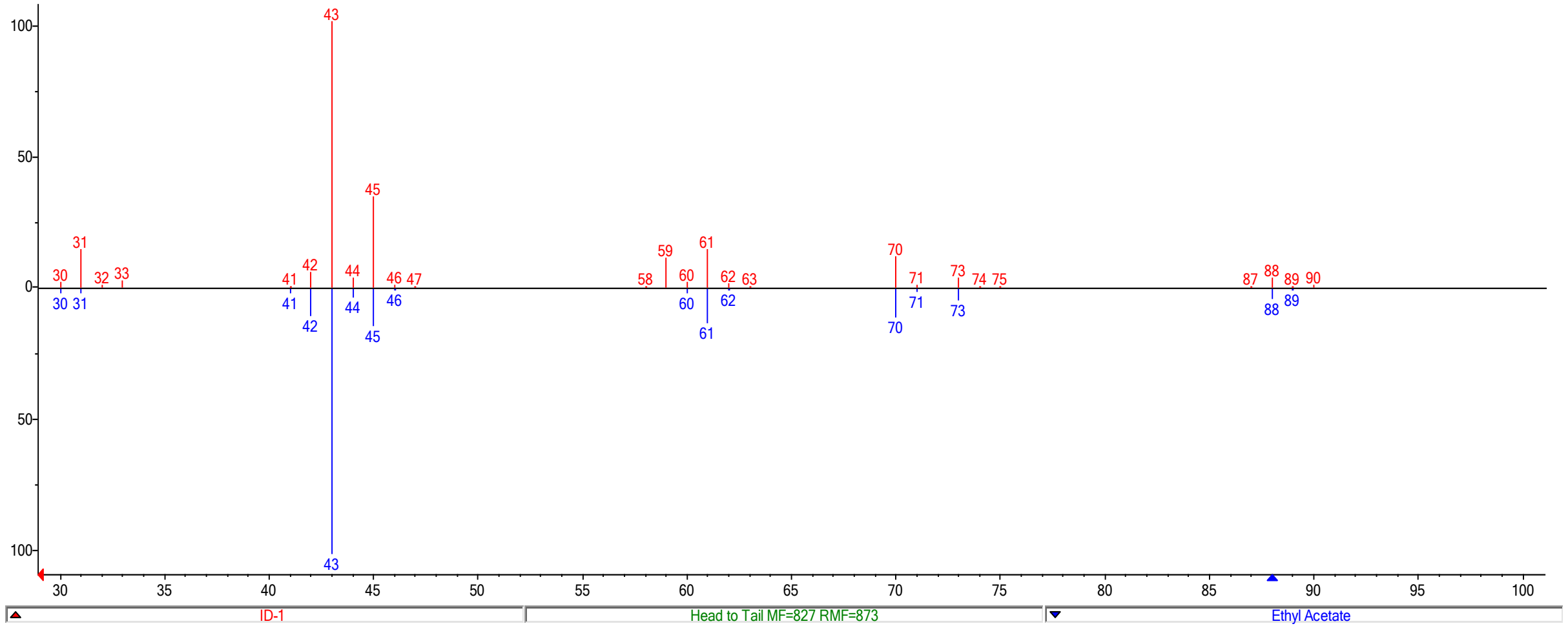
Peak # 2;

RT: 1.919 min

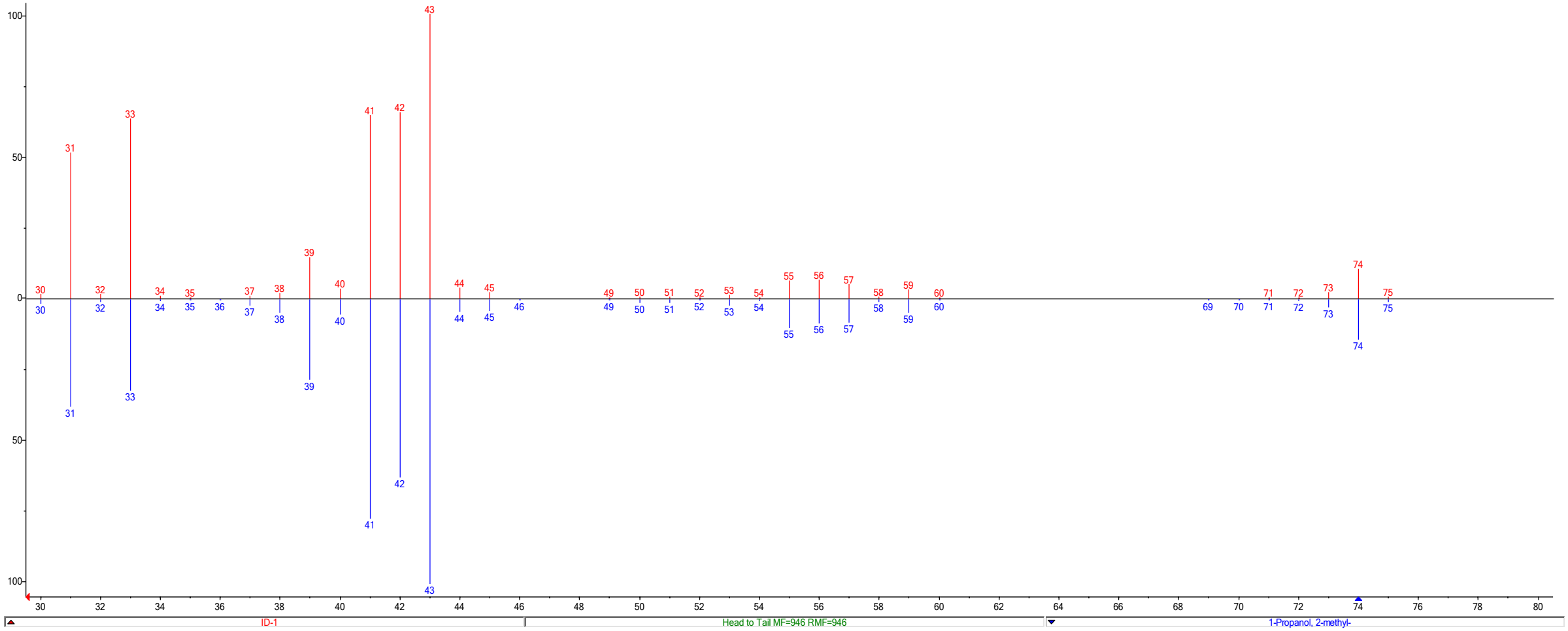
Suggested ID: 2,3-butanedione



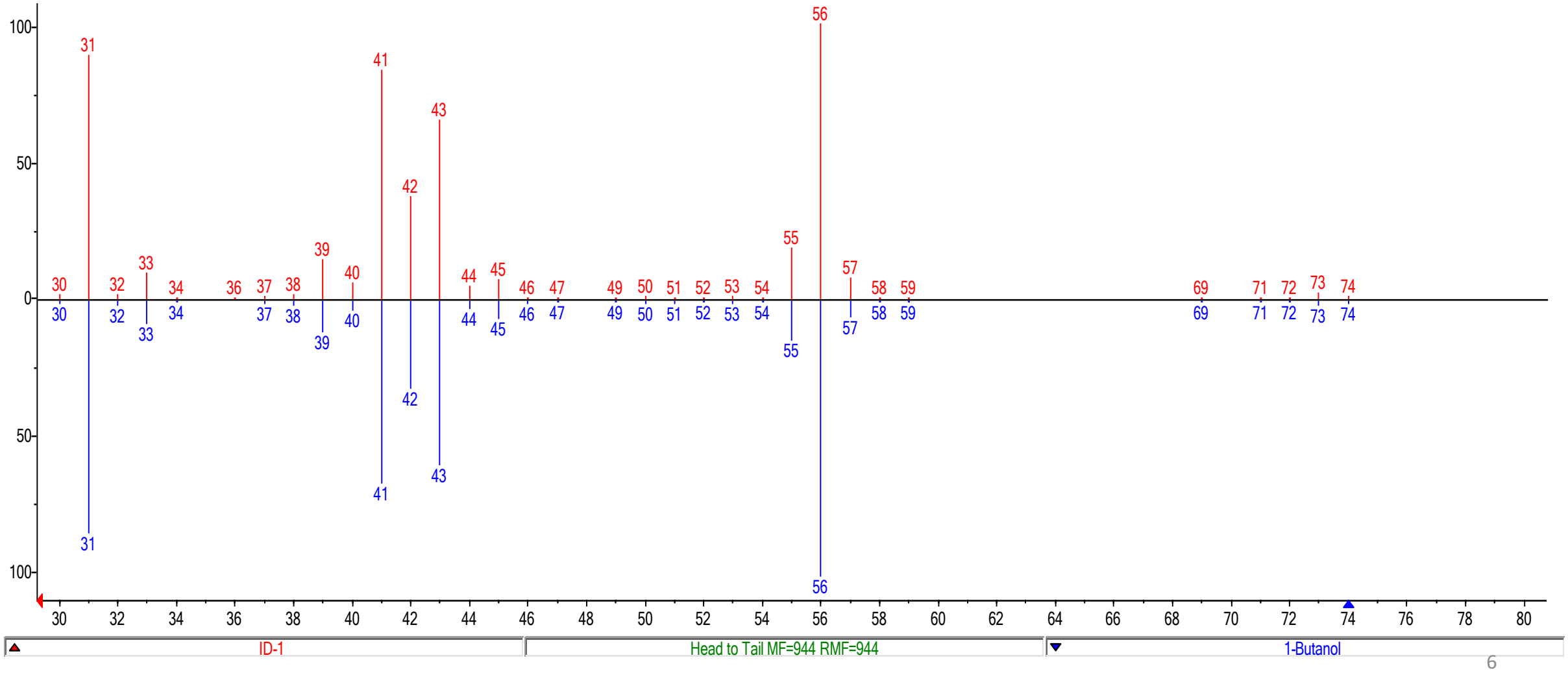
Peak # 5;
RT: 2.133 min
Suggested ID: ethyl acetate



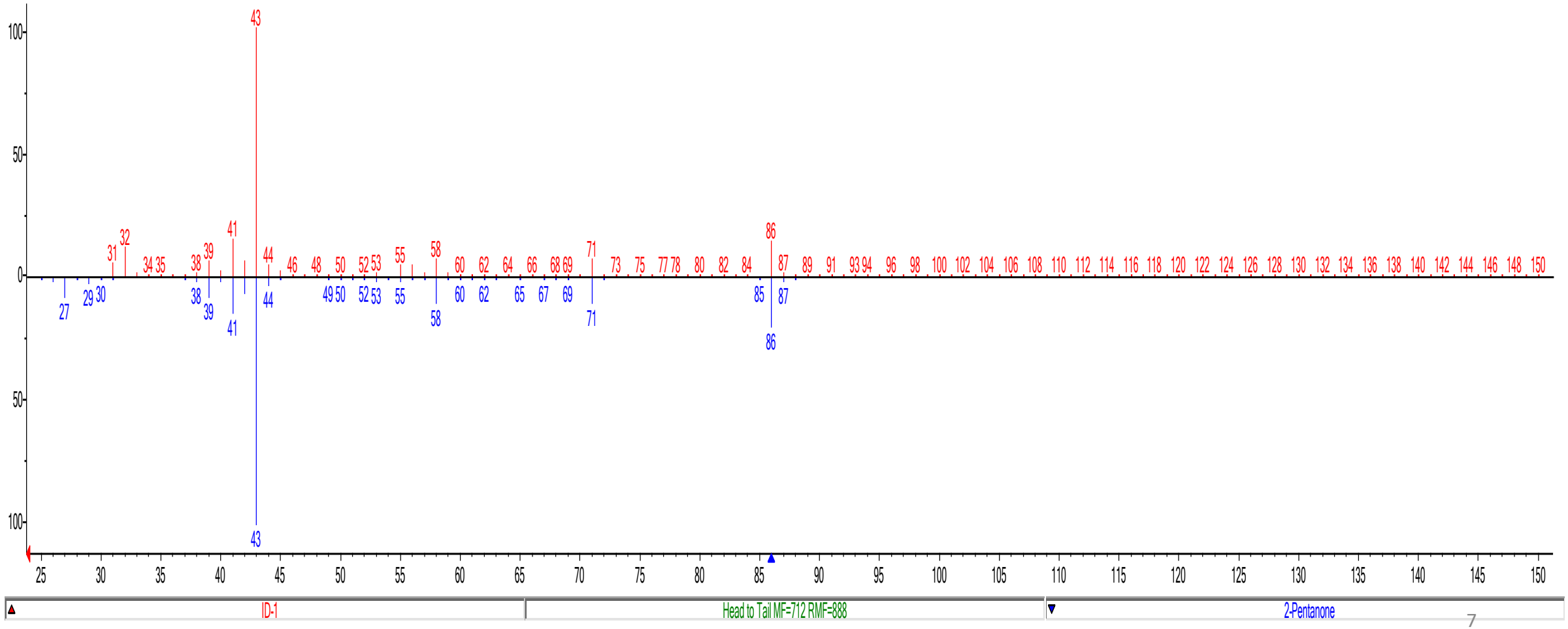
Peak # 6;
RT: 2.292 min
Suggested ID: 2-methyl 1-propanol



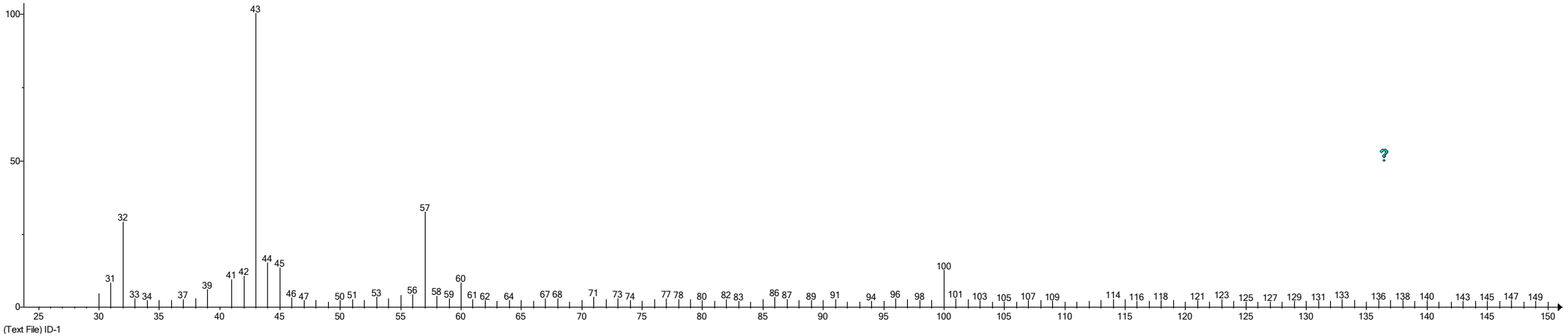
Peak # 9;
RT: 2.637 min
Suggested ID: 1-butanol

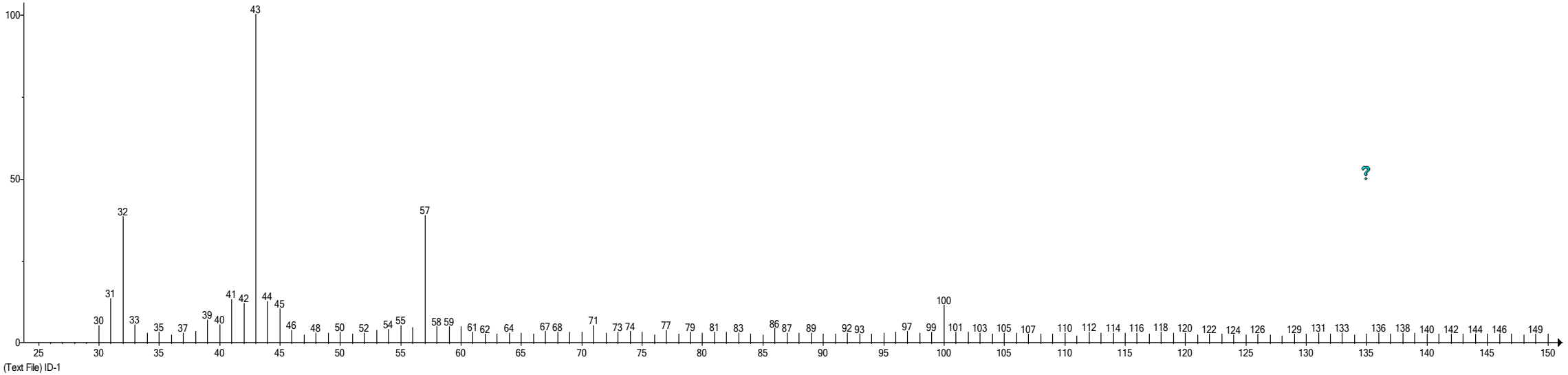


Peak # 11;
RT: 2.817 min
Suggested ID: 2-Pentanone

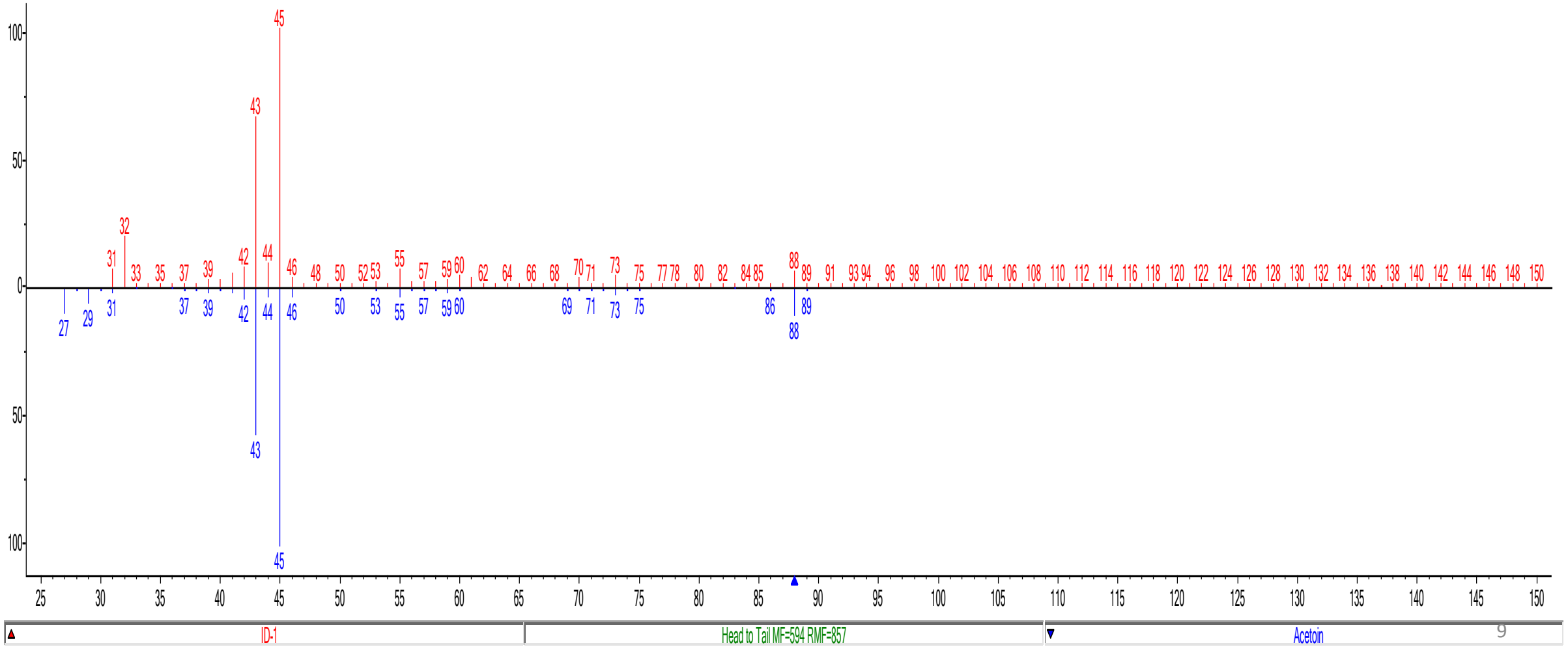


Peak # 13;
RT: 2.896 min
Suggested ID: Unknown 1

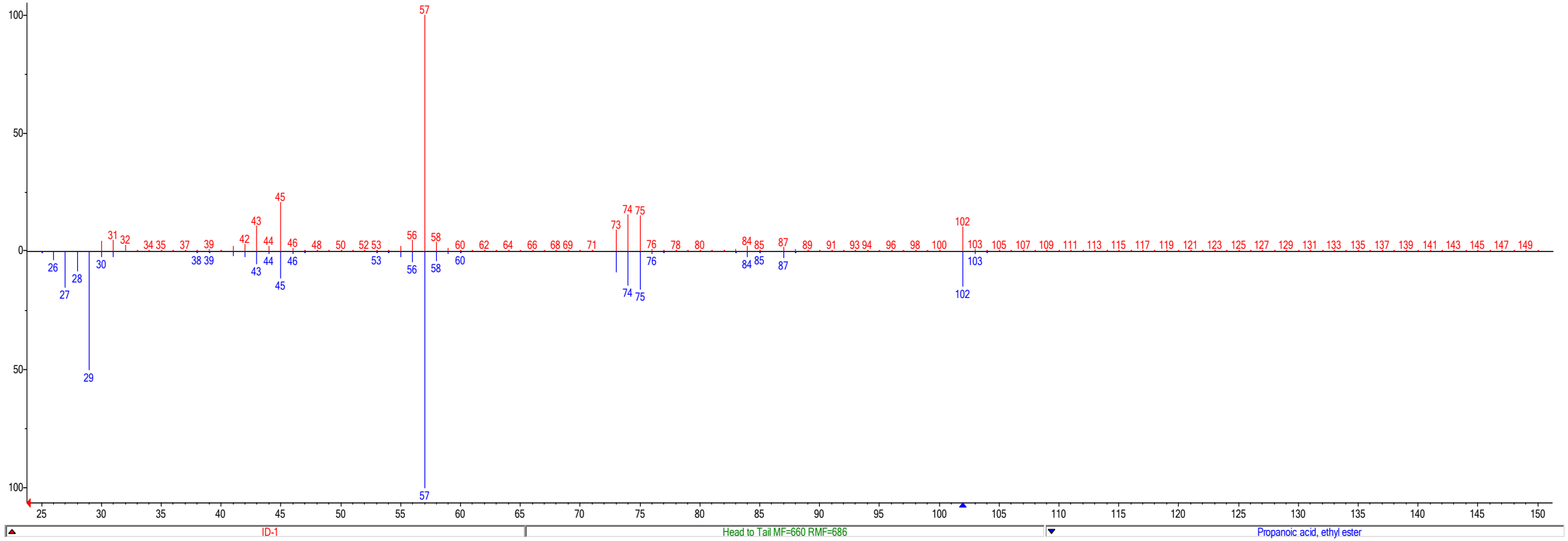




Peak # 16;
RT: 3.107 min
Suggested ID: 3-hydroxy-2-butanone



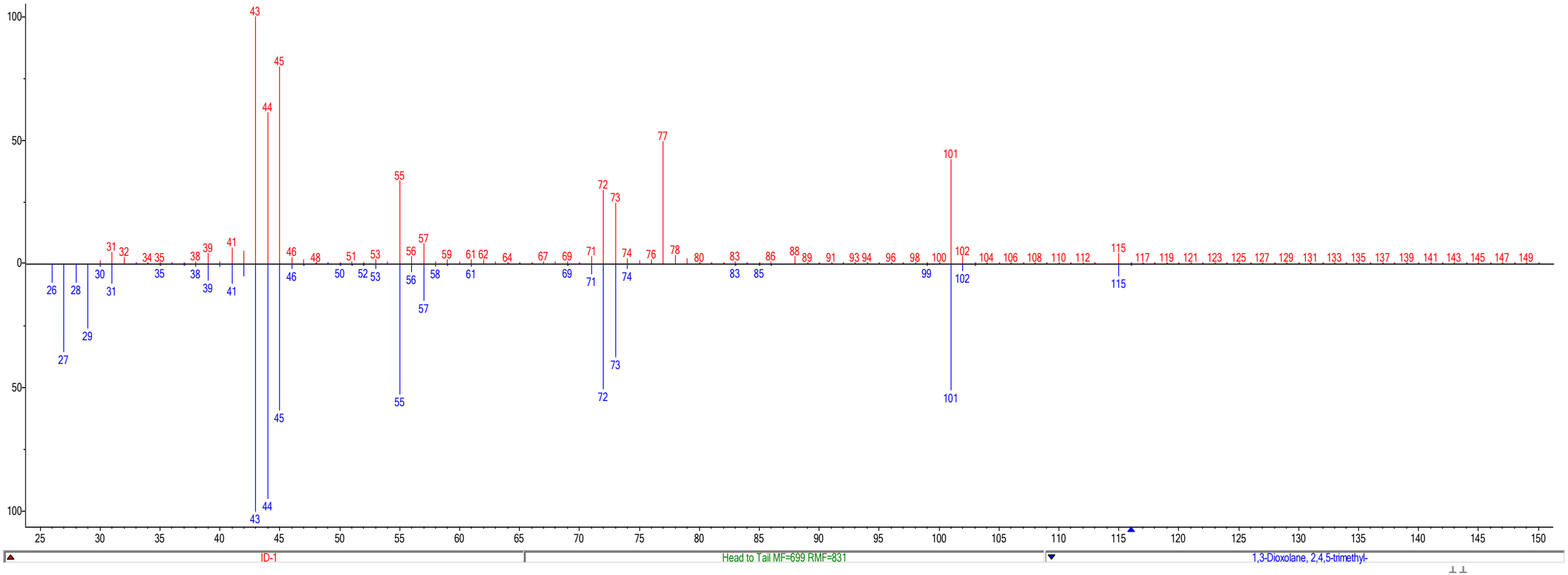
Peak # 17;
RT: 3.256 min
Suggested ID: ethyl propanoate



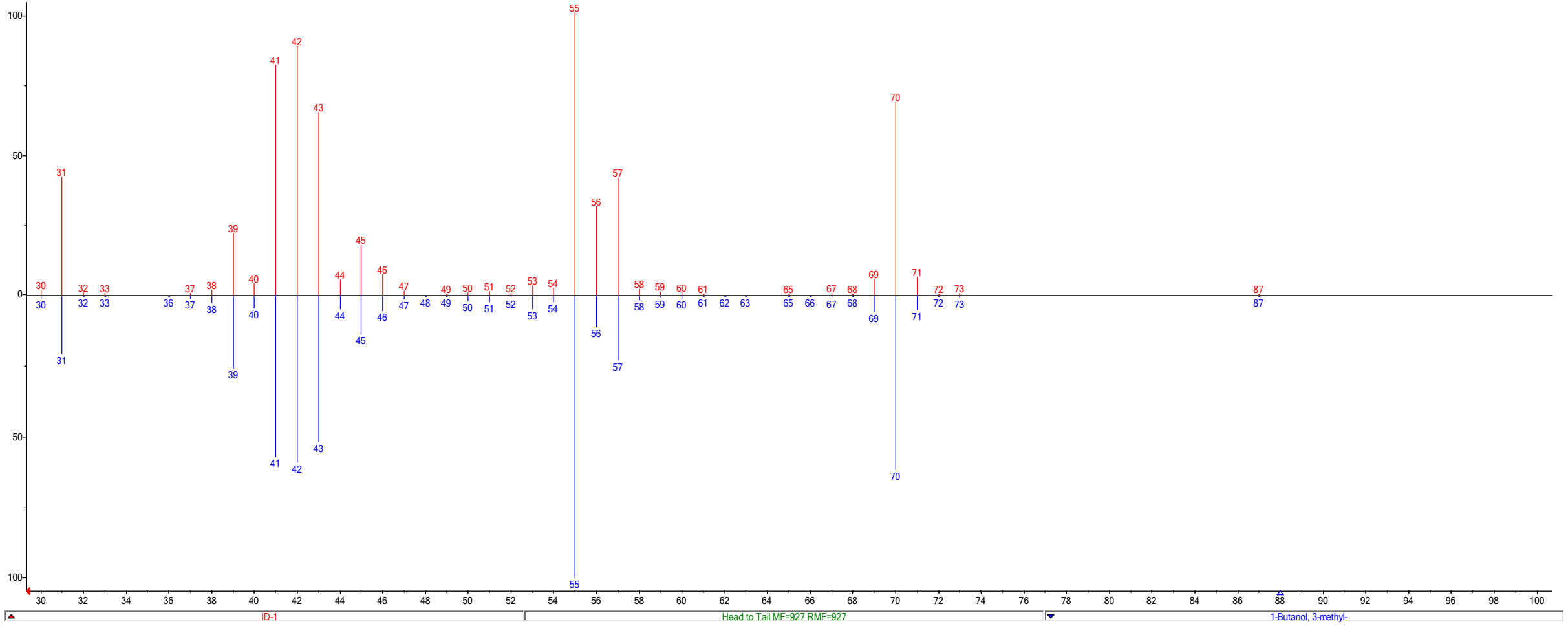
Peak # 19;

RT: 3.581 min

Suggested ID: 2,4,5-trimethyl-1,3-dioxolane (Isomer A)



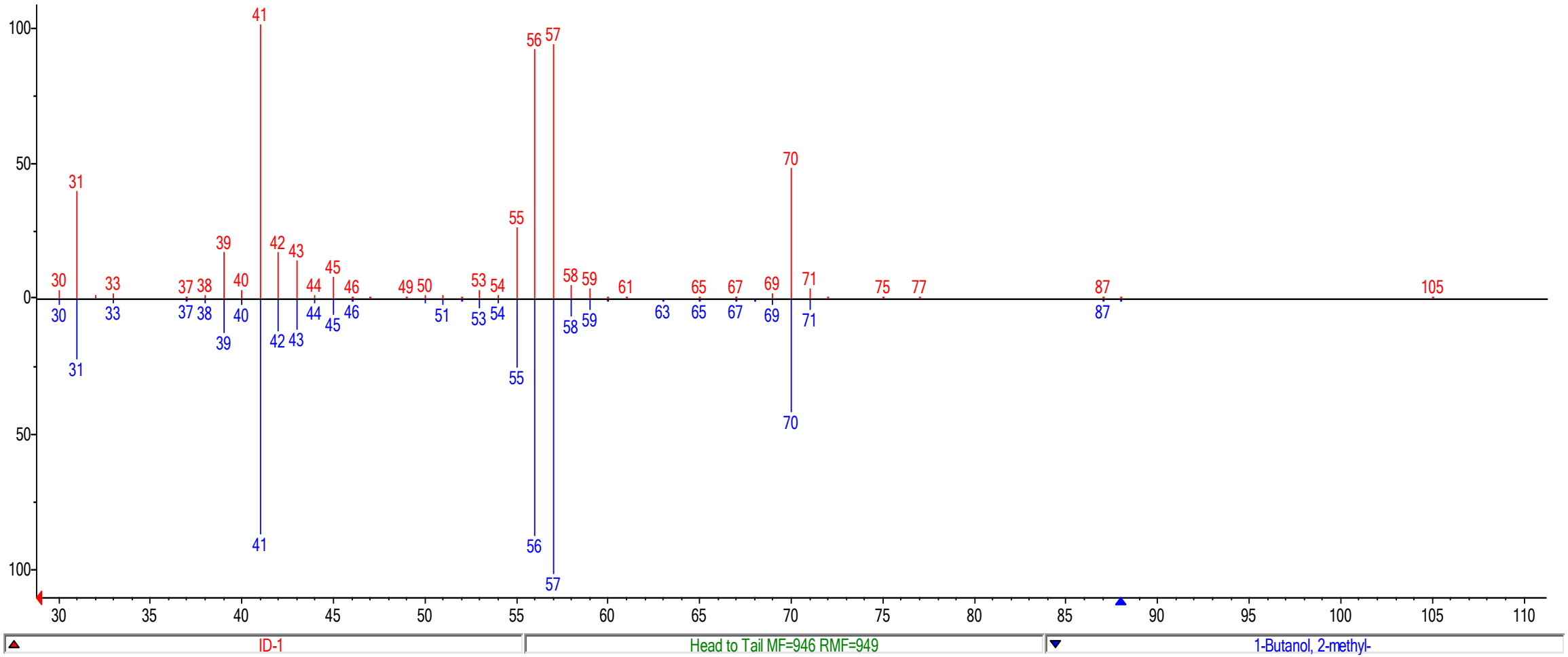
Peak # 20;
RT: 3.960 min
Suggested ID: 3-methyl butanol (Isoamyl alcohol)



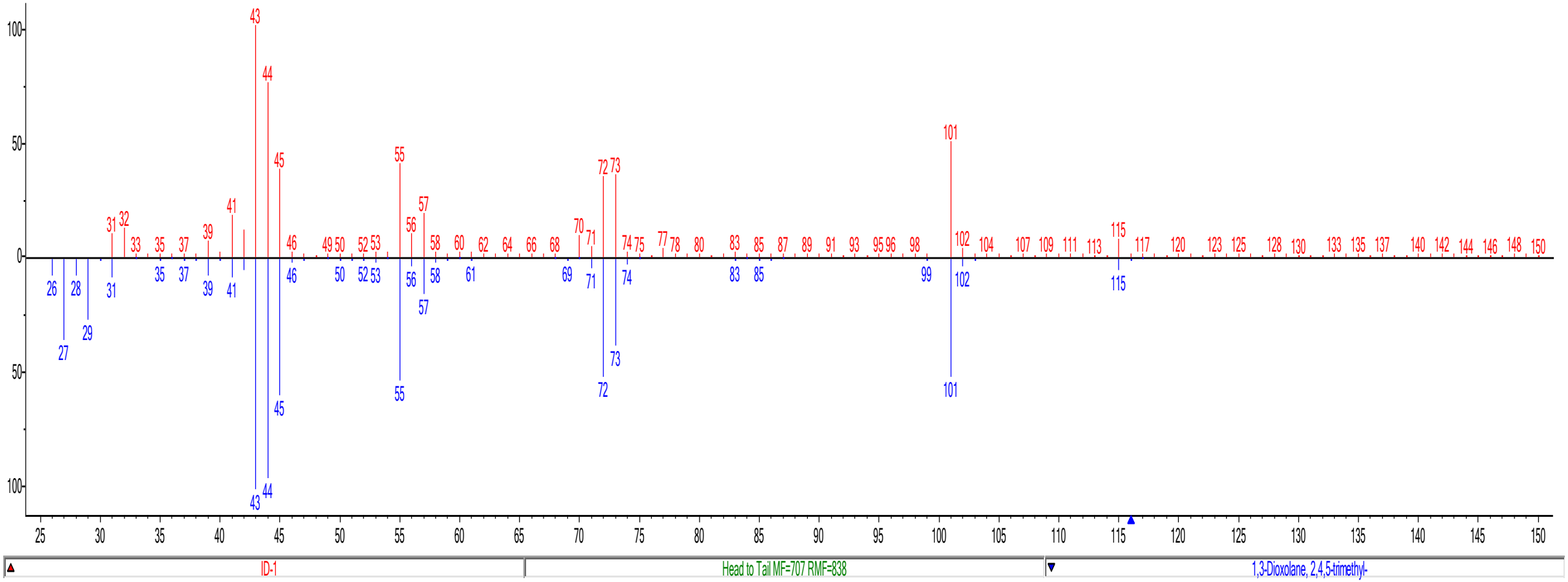
Peak # 21;

RT: 3.990 min

Suggested ID: 2-methylbutyl alcohol (amyl alcohol)



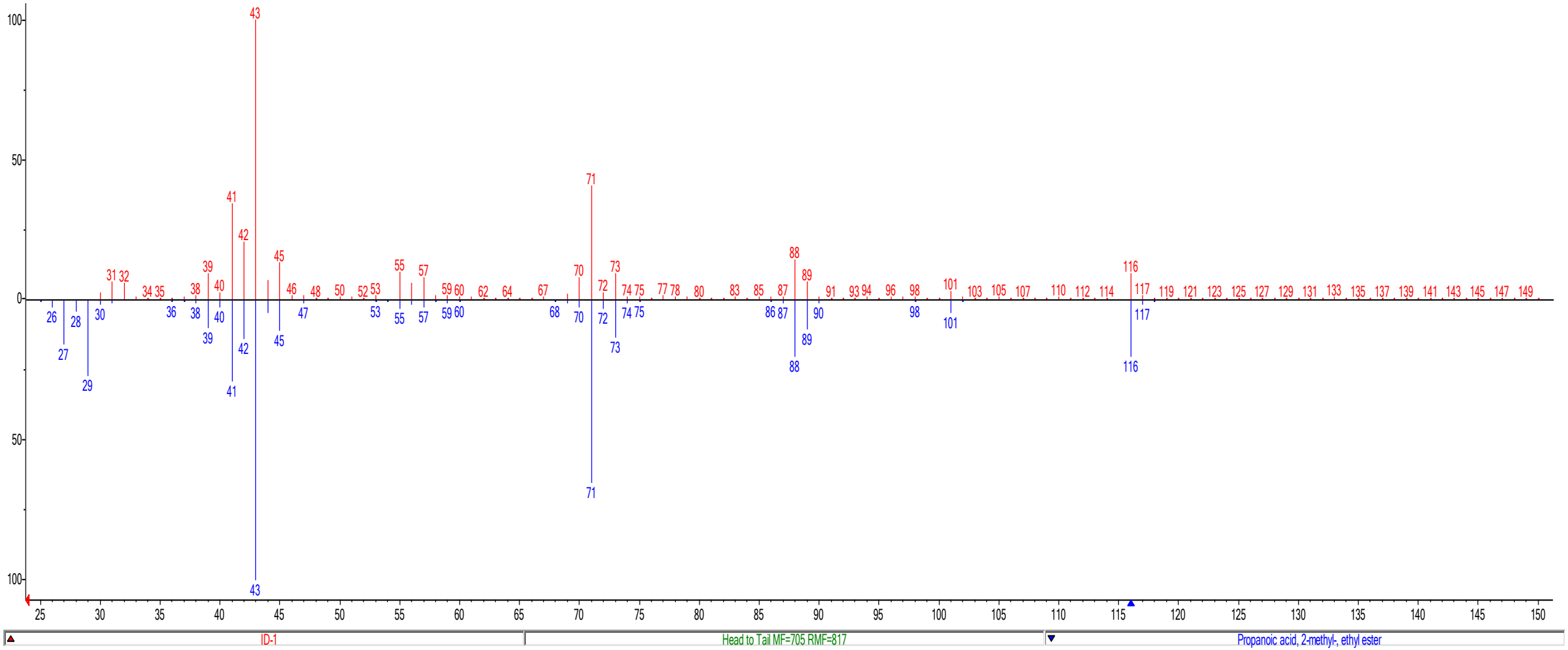
Peak # 22;
RT: 4.109 min
Suggested ID: 2,4,5-trimethyl-1,3-dioxolane (Isomer B)



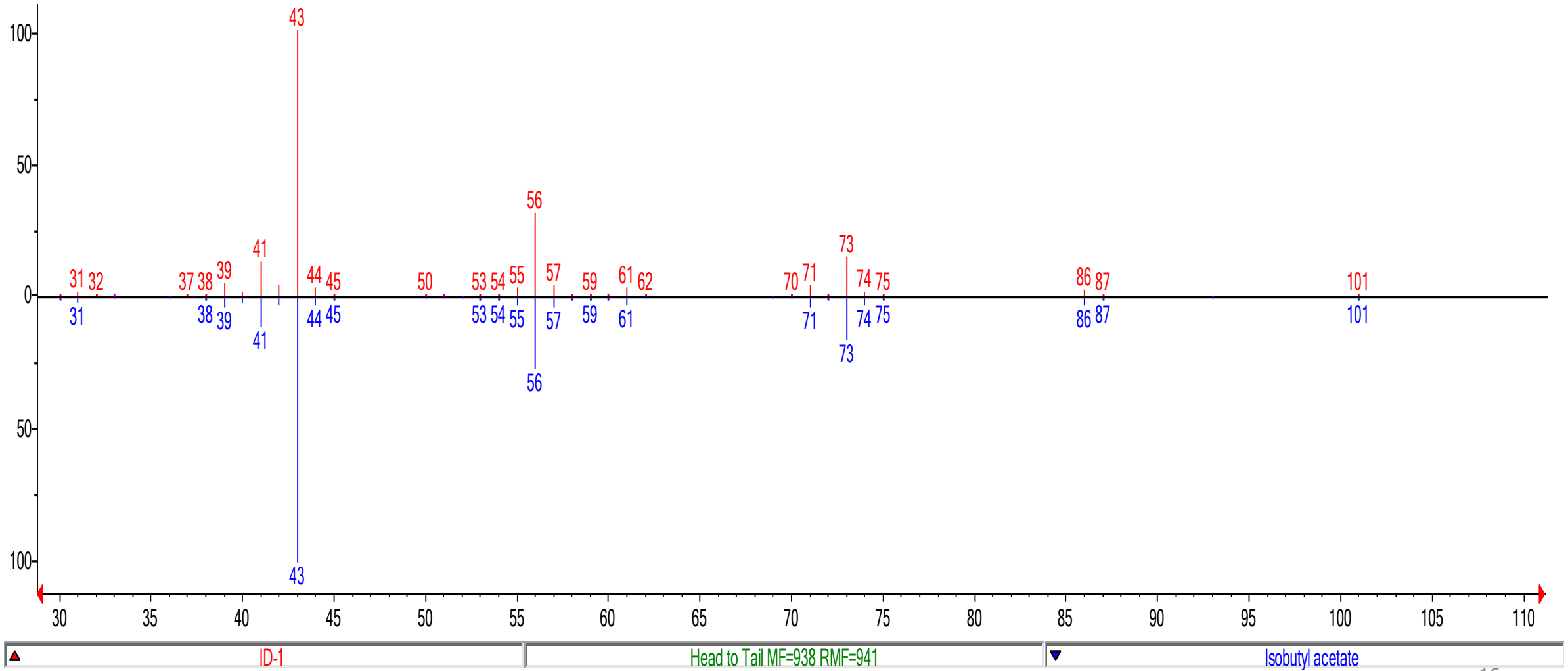
Peak # 25

RT: 4.274 min

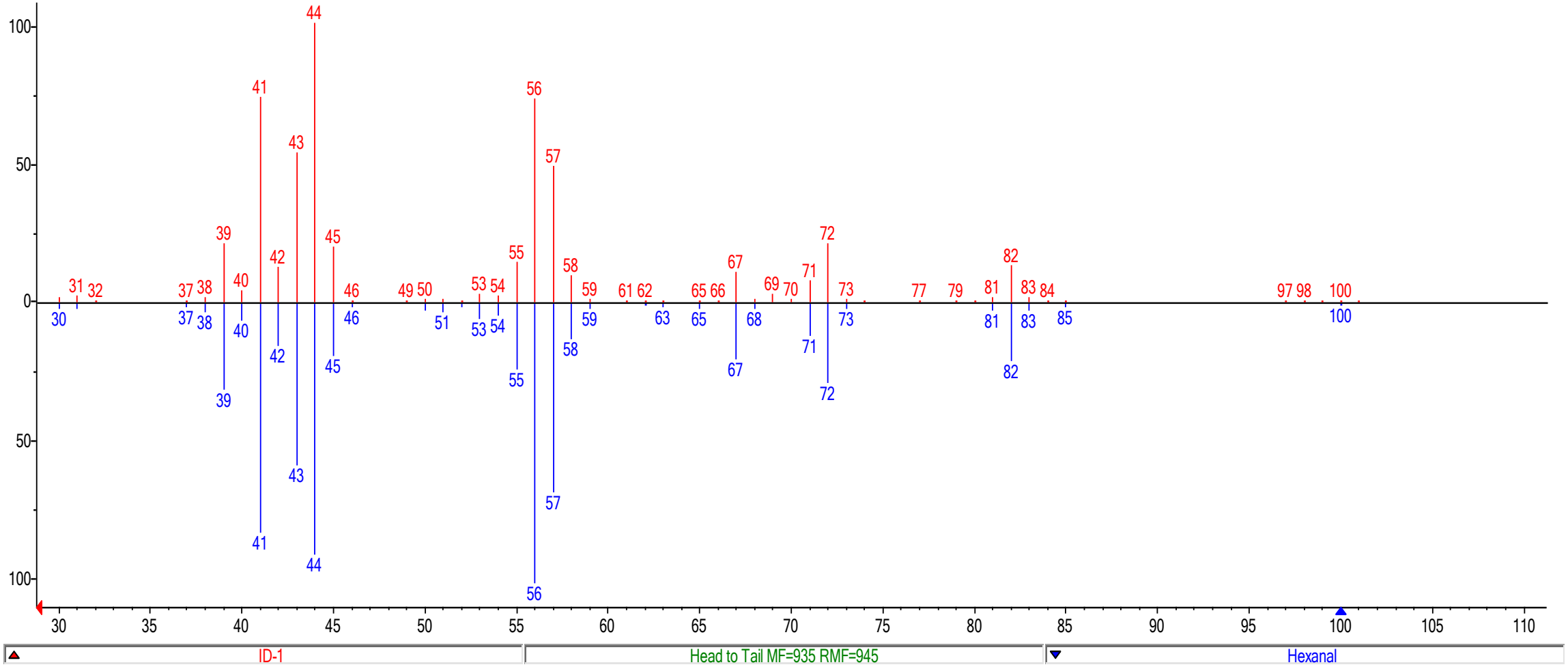
Suggested ID: 2-methylethyl propanoate



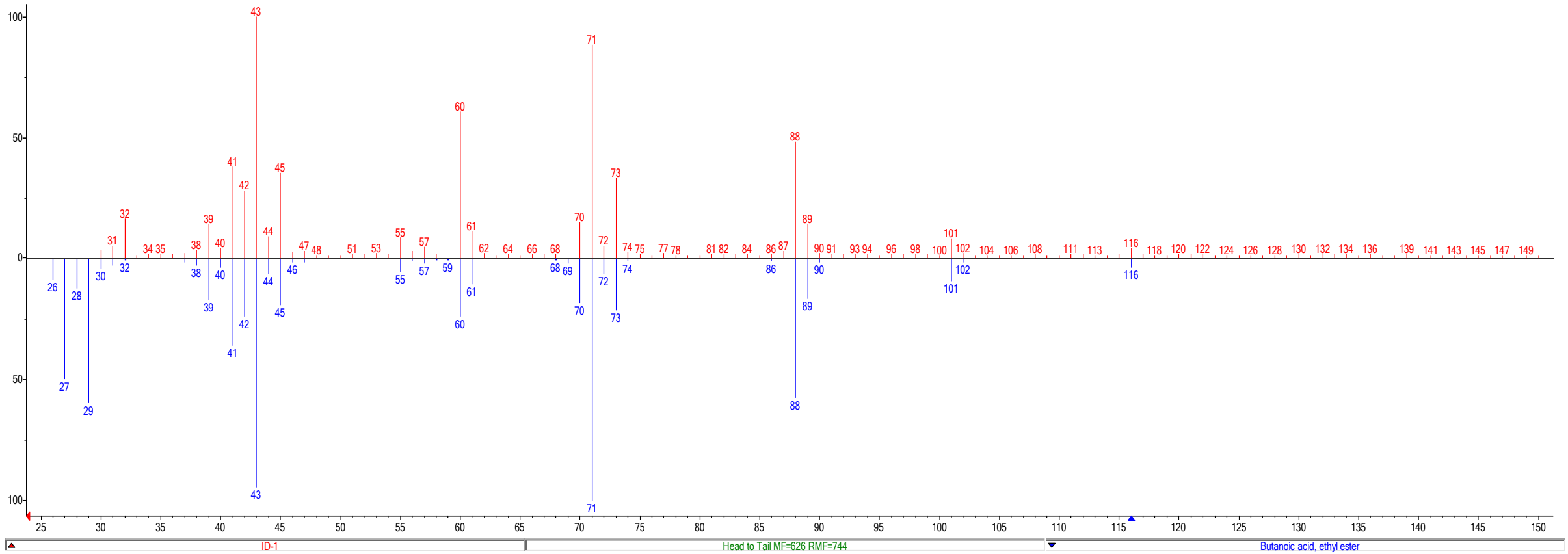
Peak # 27;
RT: 4.573 min
Suggested ID: 2-methylproyl acetate



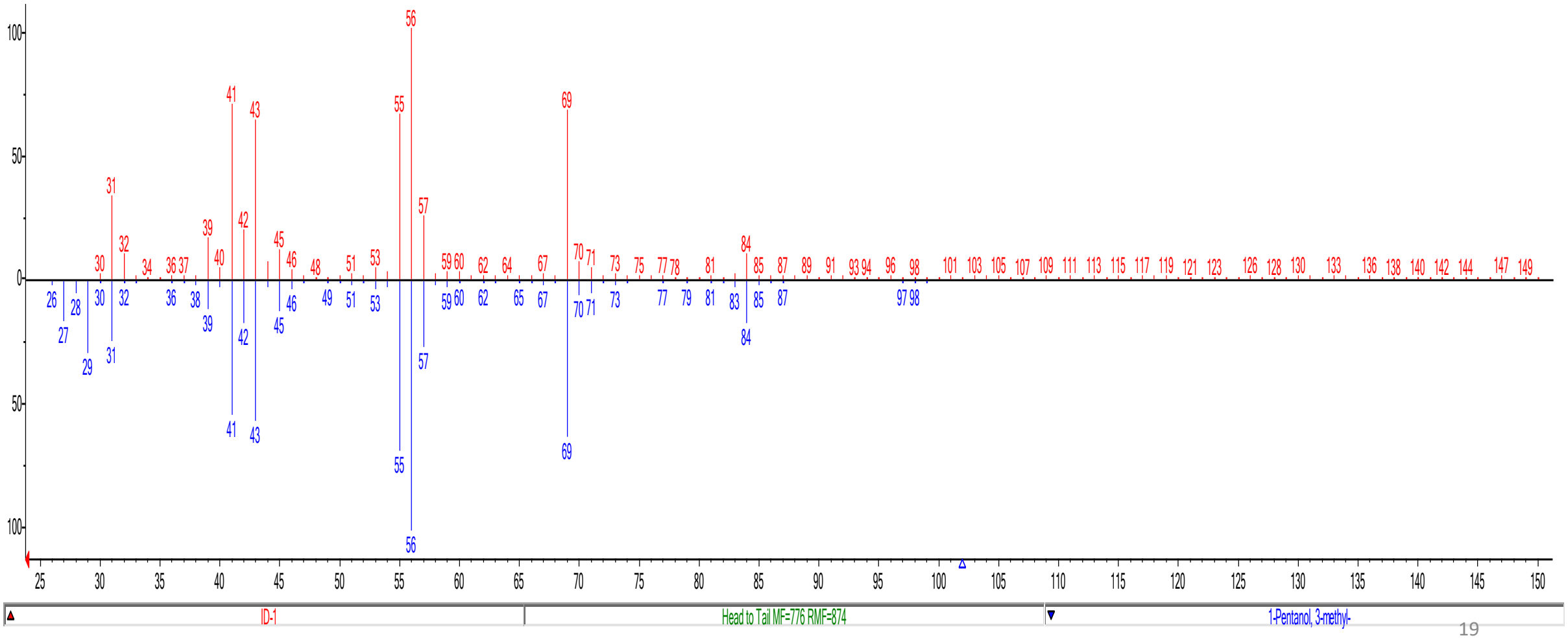
Peak # 30;
RT: 5.038 min
Suggested ID: Hexanal



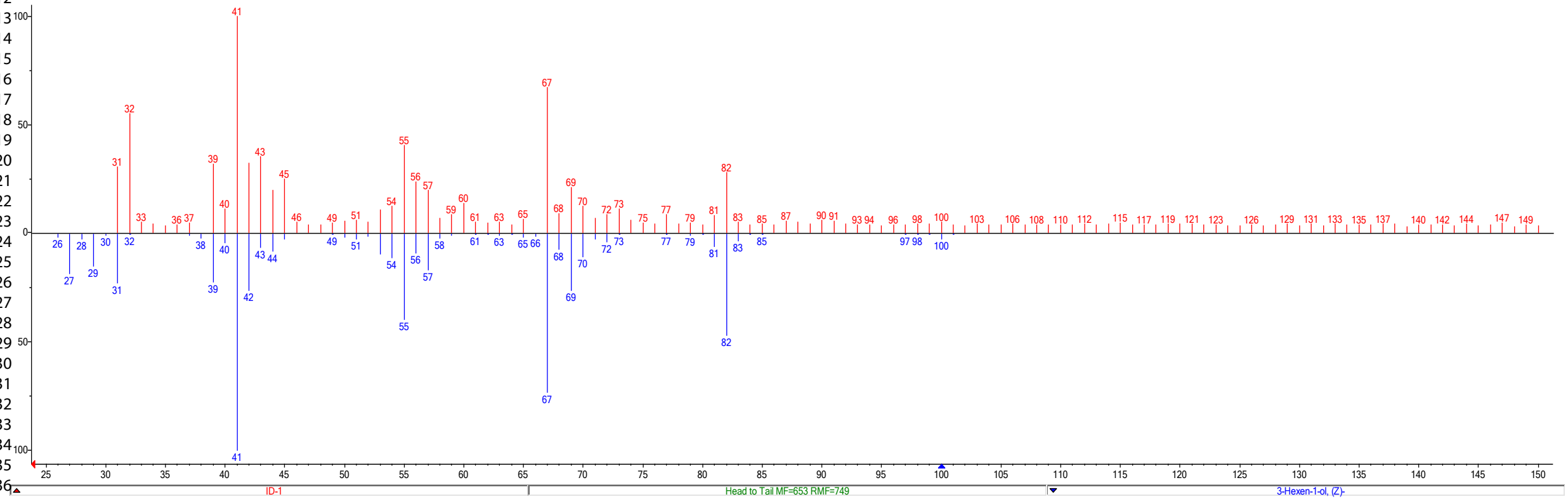
Peak # 31;
RT: 5.173 min
Suggested ID: ethyl butanoate



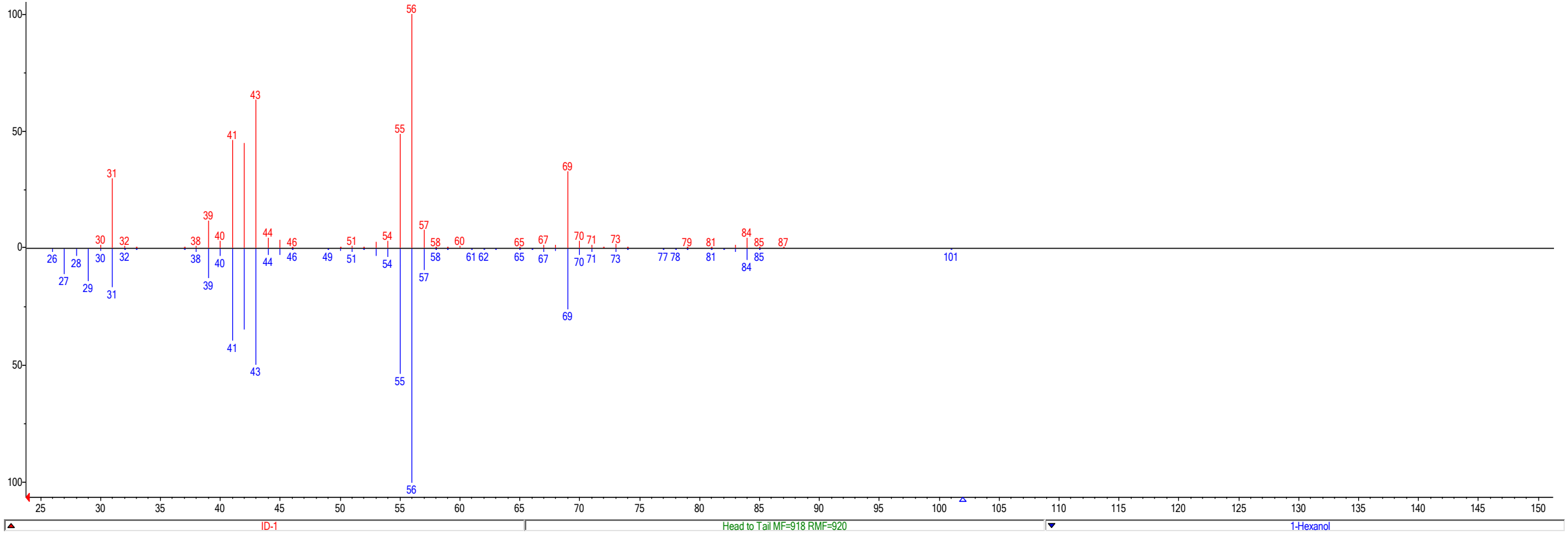
Peak # 39;
RT: 6.719 min
Suggested ID: 3-methyl-1-pentanol



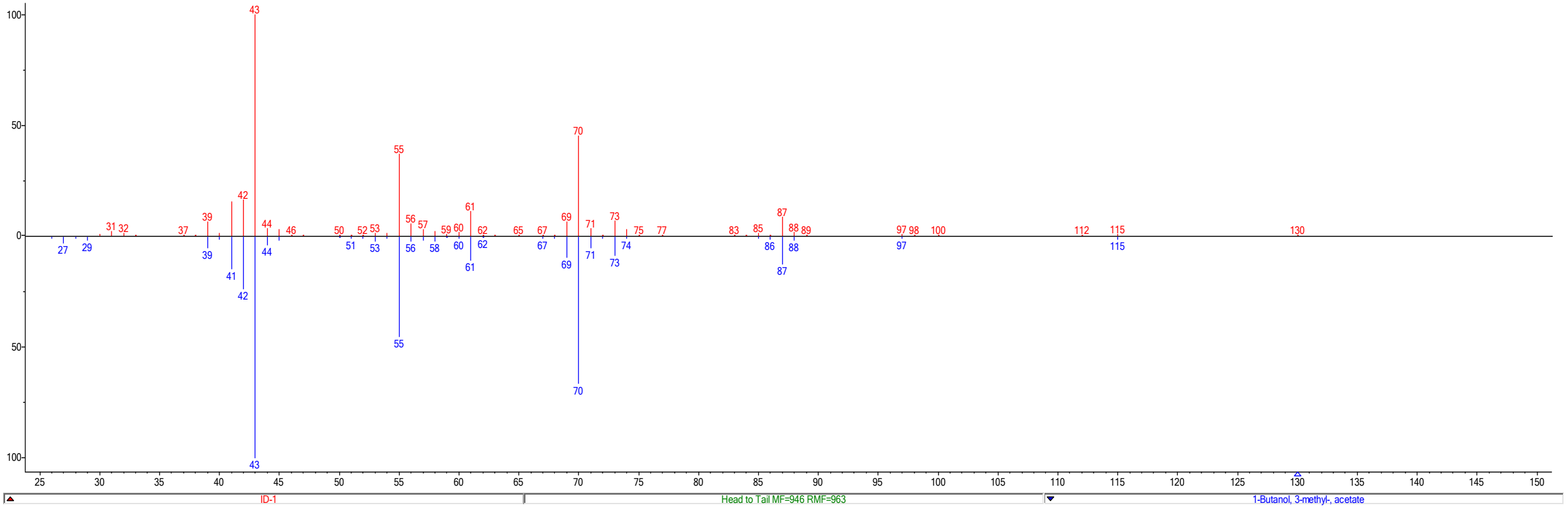
Peak # 42;
RT: 7.022 min
Suggested ID: 3-Hexen-1-ol



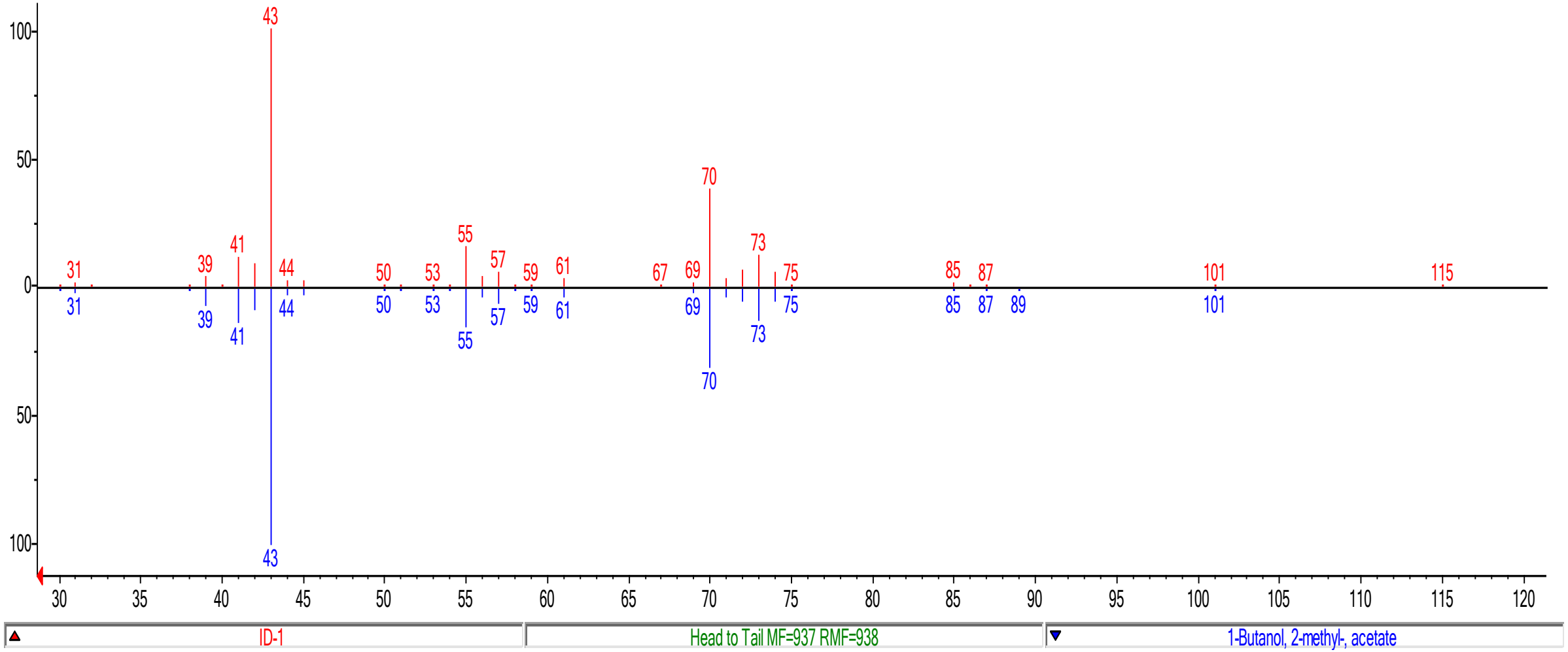
Peak # 44;
RT: 7.627 min
Suggested ID: 1-hexanol



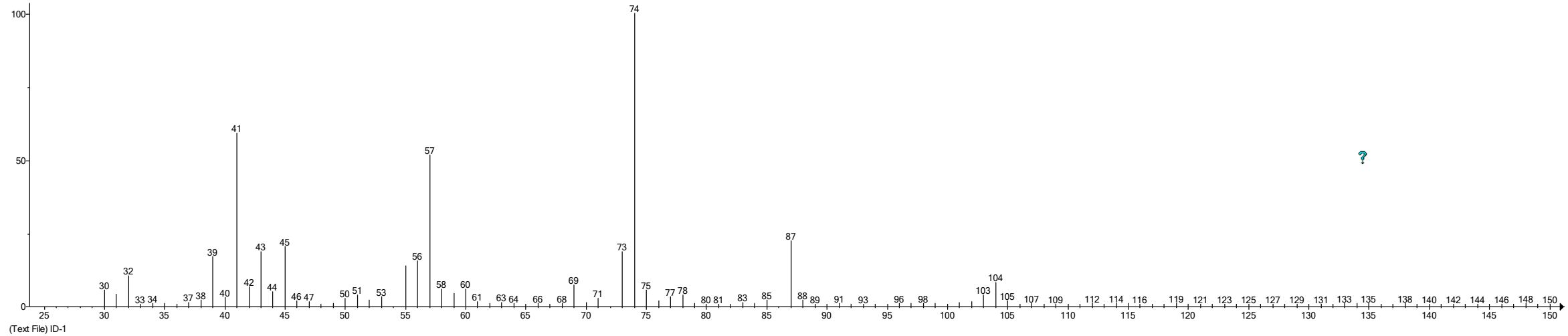
Peak # 45;
RT: 7.784 min
Suggested ID: 3-methylbutyl acetate
(Isoamyl acetate)

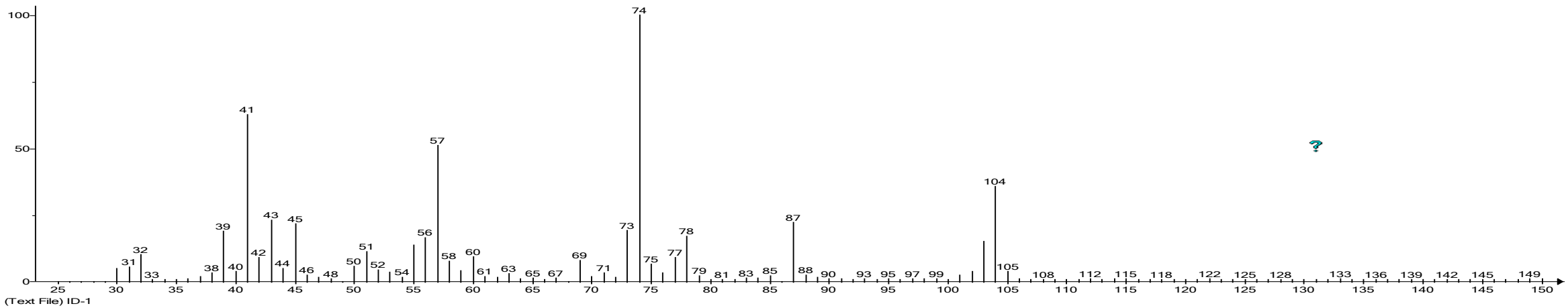


Peak # 46;
RT: 7.892 min
Suggested ID: 2-methylbutyl acetate

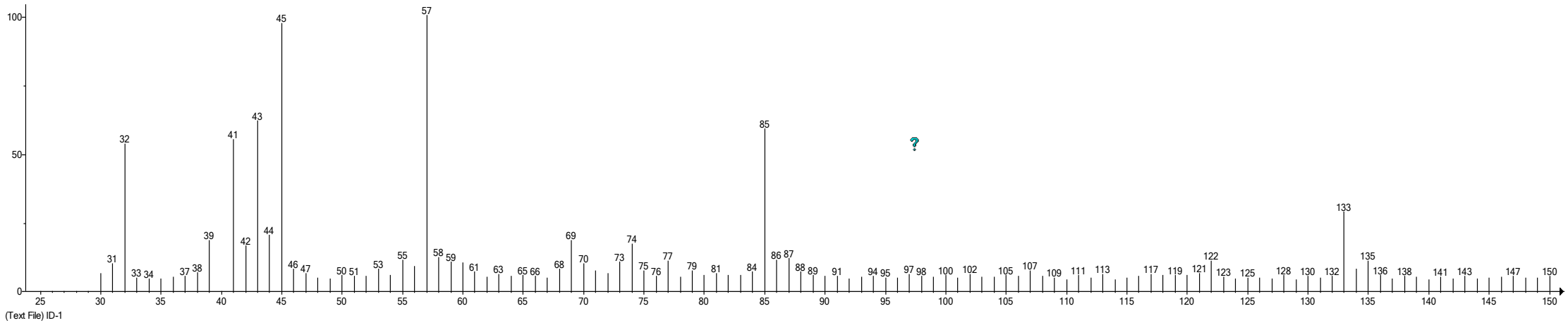


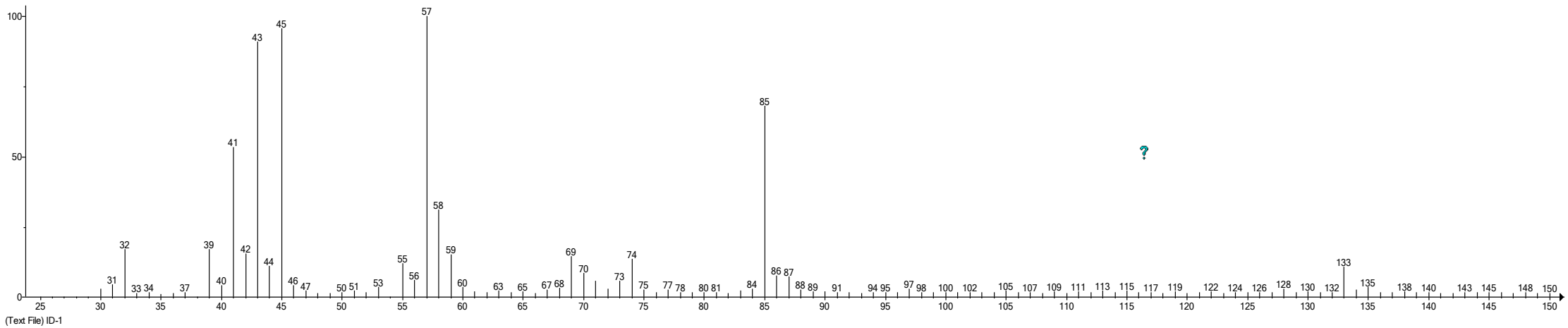
Peak # 50;
RT: 8.122 min
Suggested ID: Unknown 2





Peak # 61;
RT: 10.146 min
Suggested ID: Unknown 3

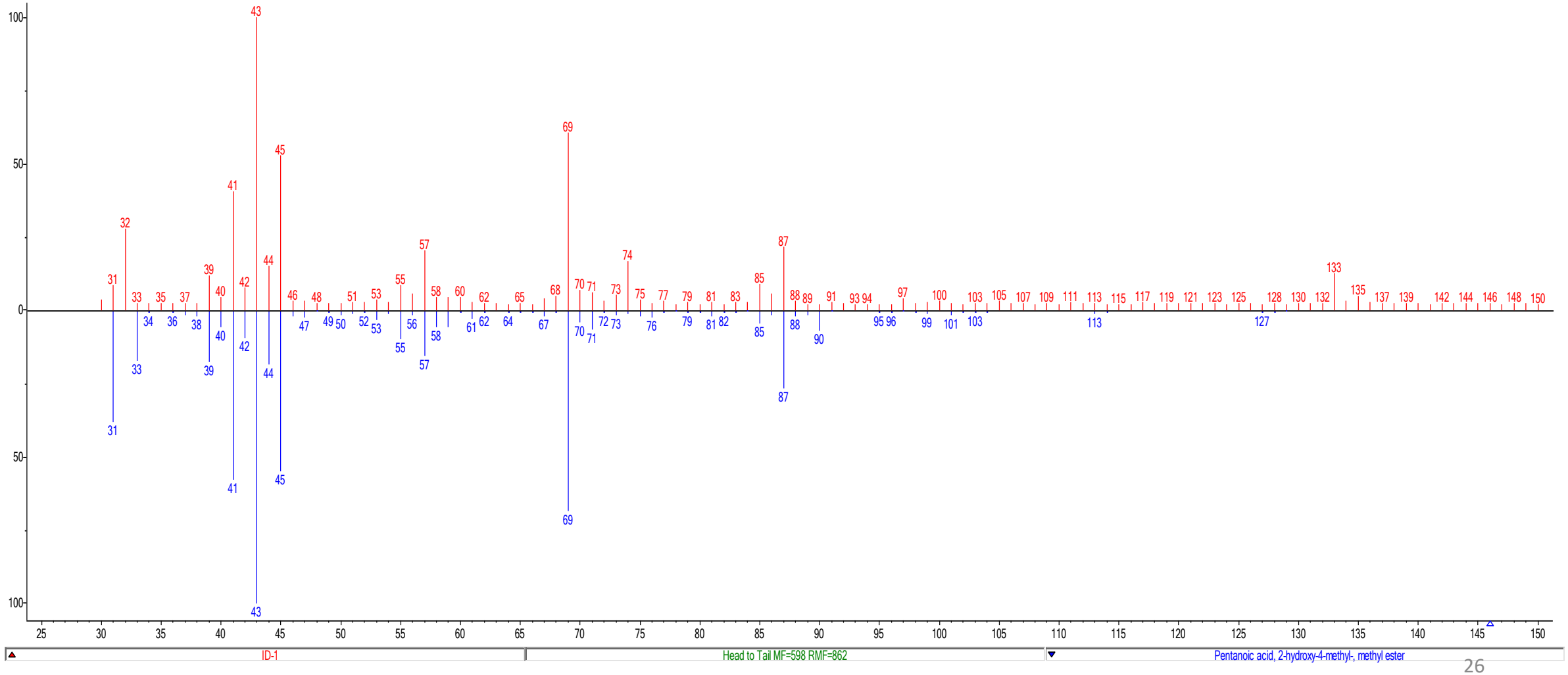




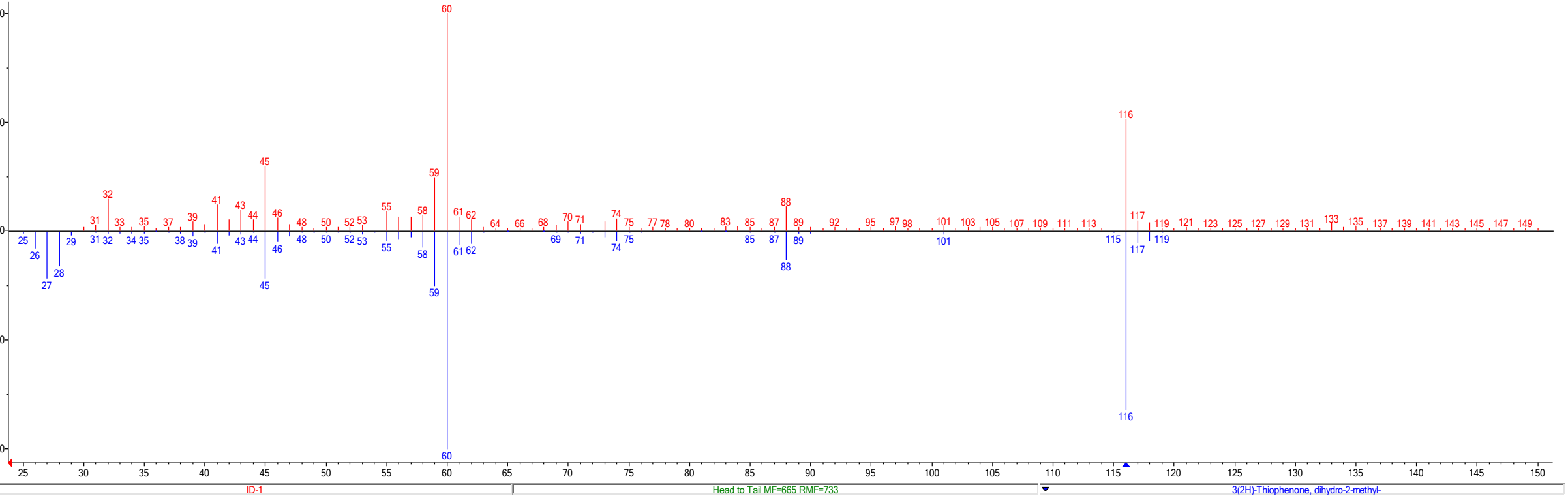
Peak # 62;

RT: 10.310 min

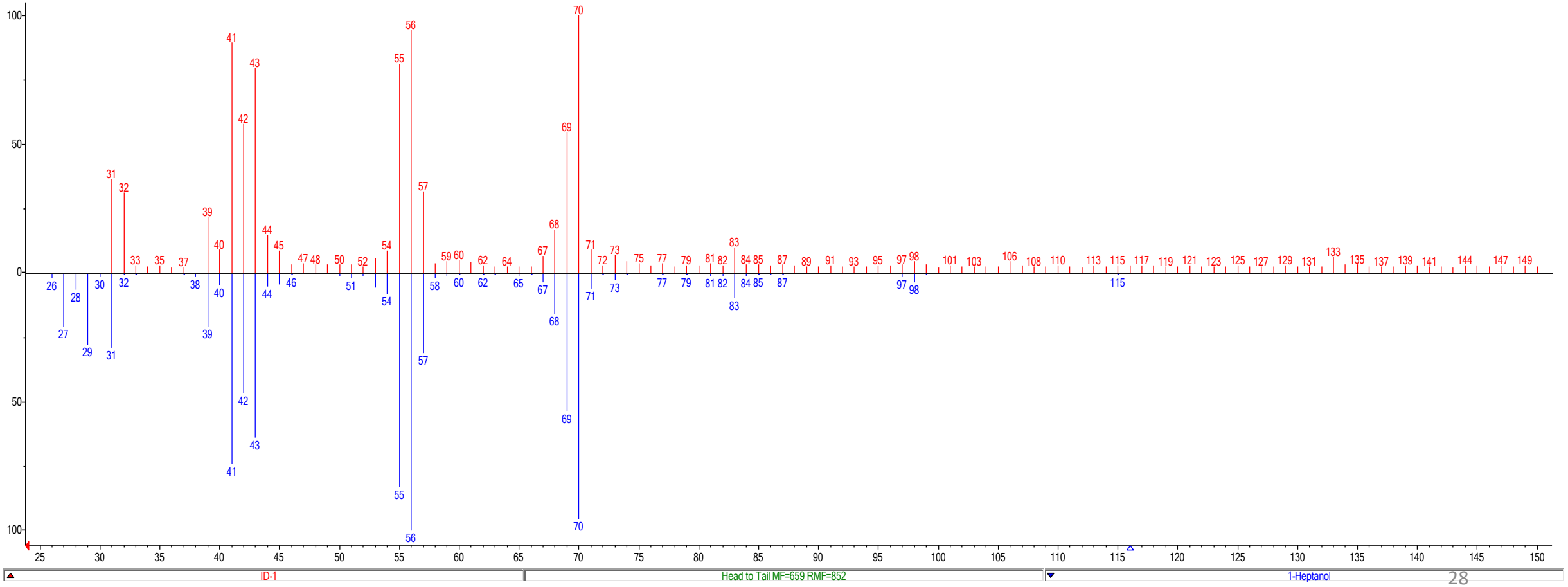
Suggested ID: methyl 2-hydroxy-4-methyl pentanoate



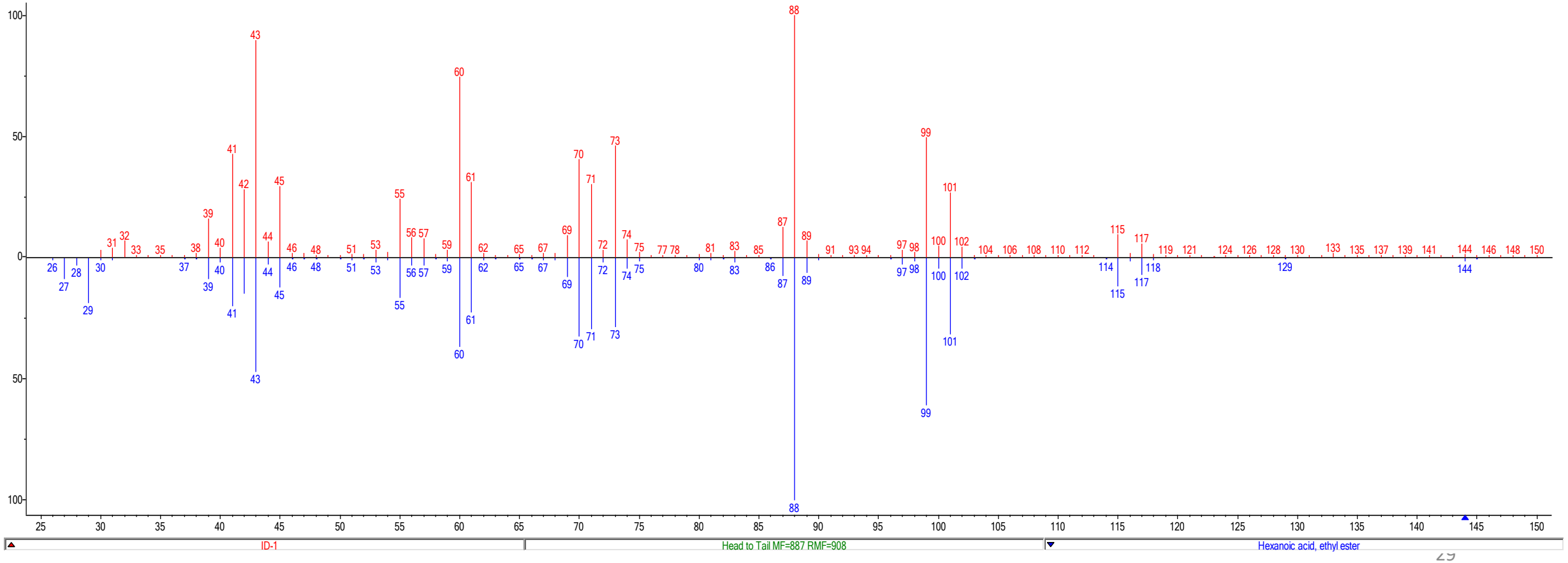
Peak # 65;
RT: 11.256 min
Suggested ID: dihydro-2-methyl-3(2H)-thiophenone
(blackberry thiophenone)



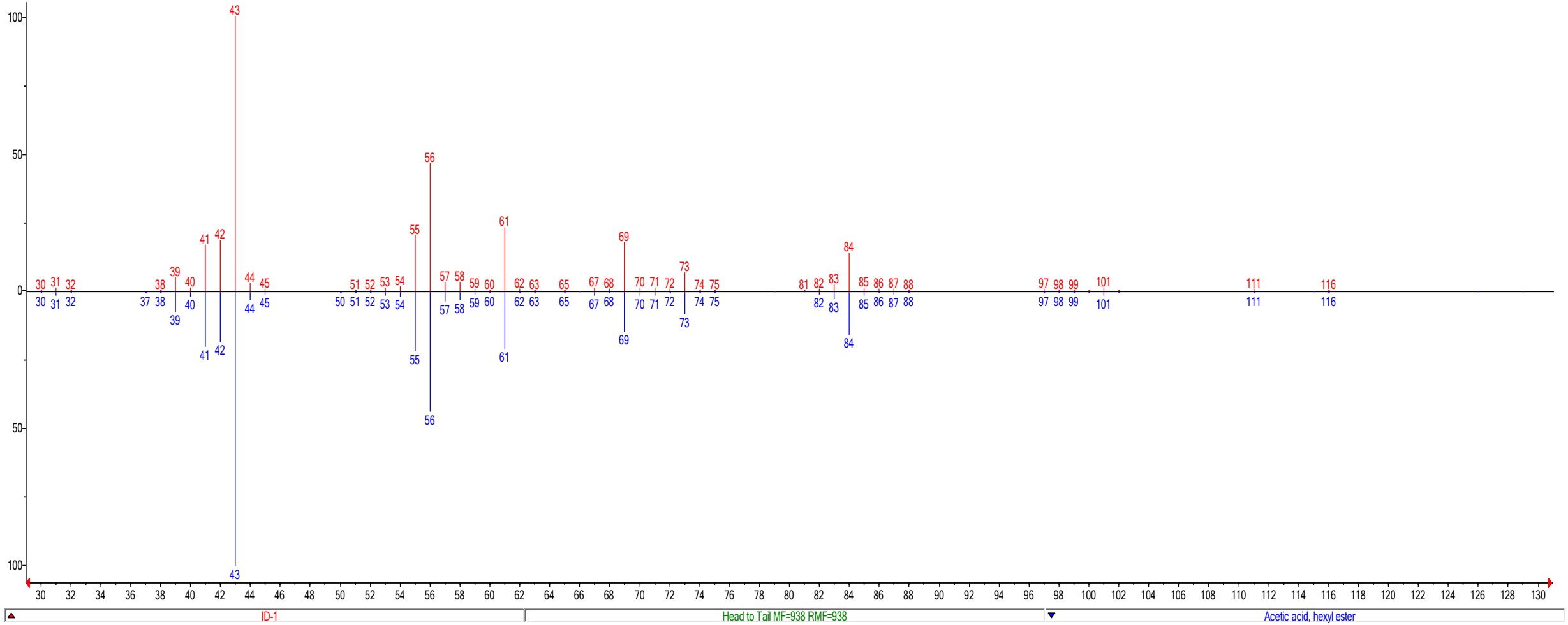
Peak # 69;
RT: 11.830 min
Suggested ID: 1-heptanol



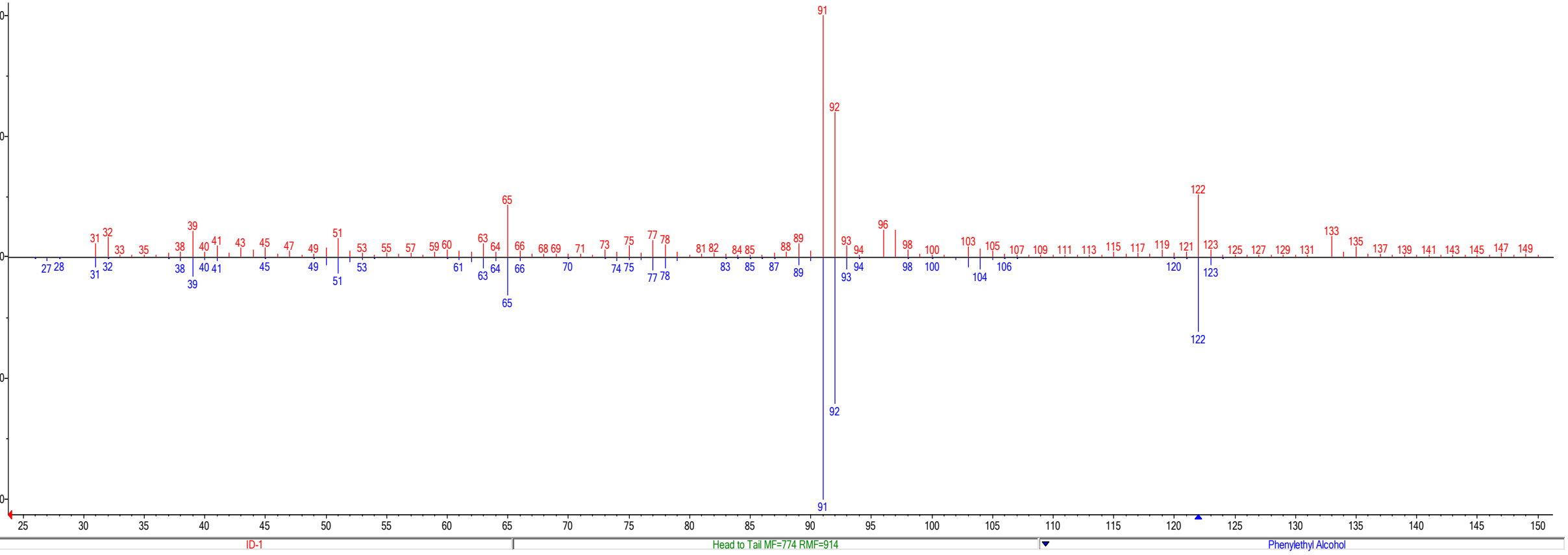
Peak # 77;
RT: 13.107 min
Suggested ID: ethyl hexanoate



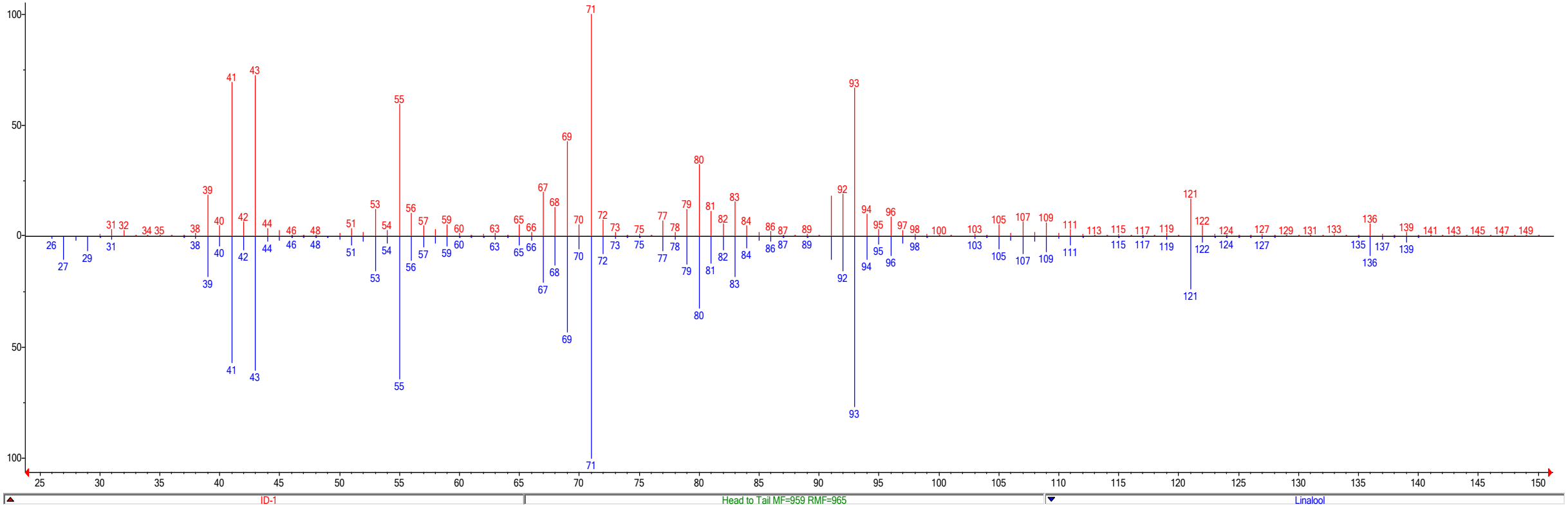
Peak # 80;
RT: 13.747 min
Suggested ID: hexyl acetate



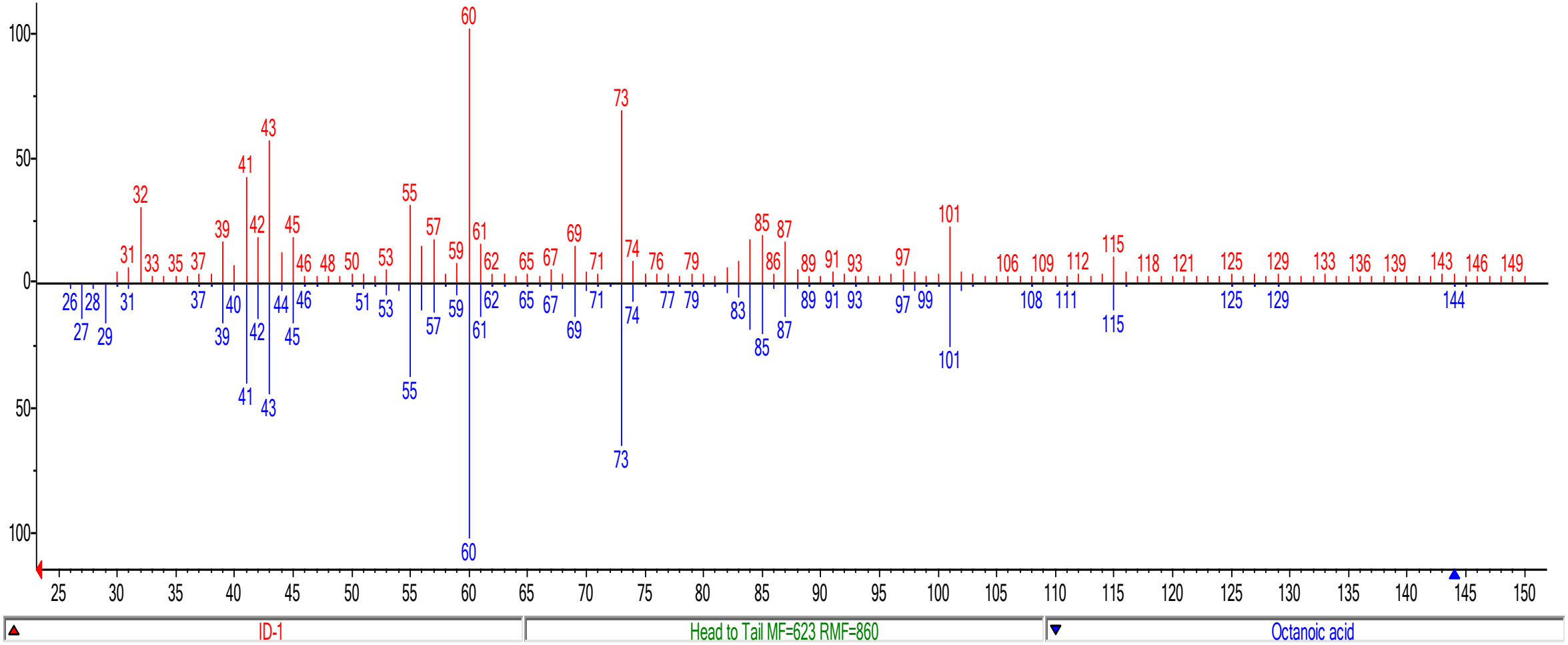
Peak # 94;
RT: 17.760 min
Suggested ID: 2-phenylethanol



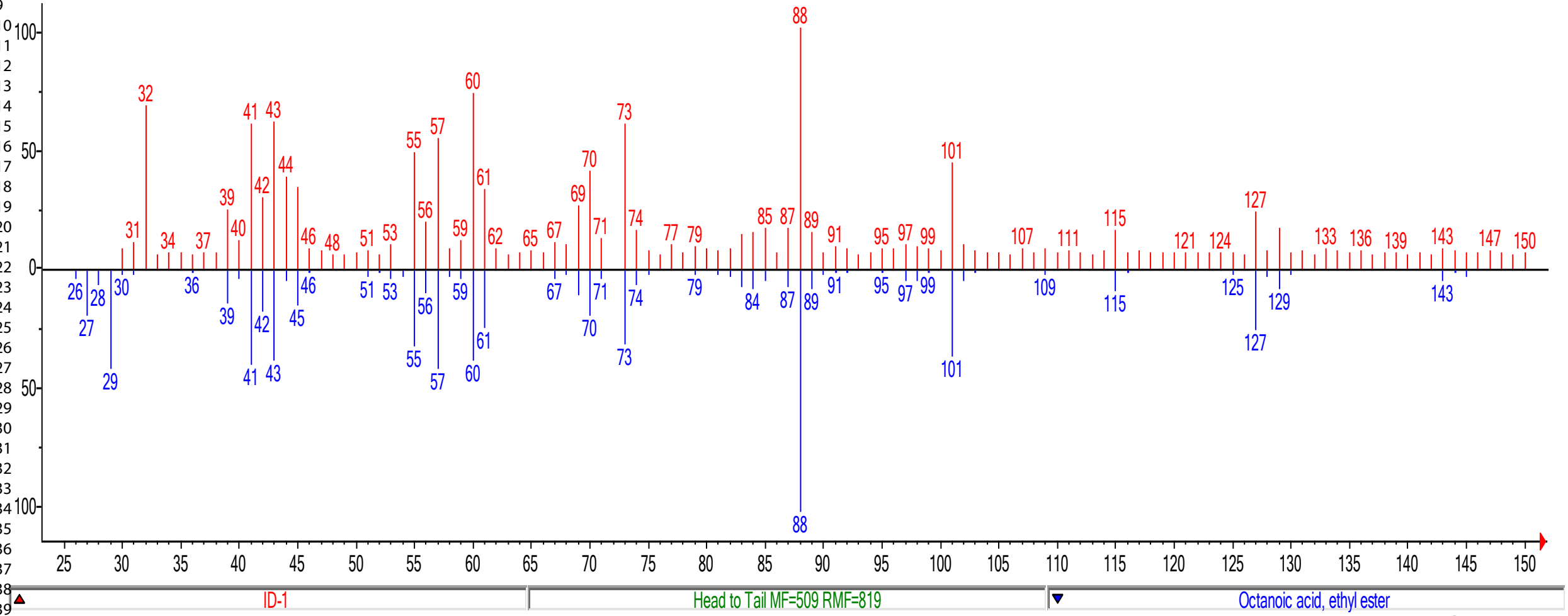
Peak # 96;
RT: 17.903 min
Suggested ID: Linalool



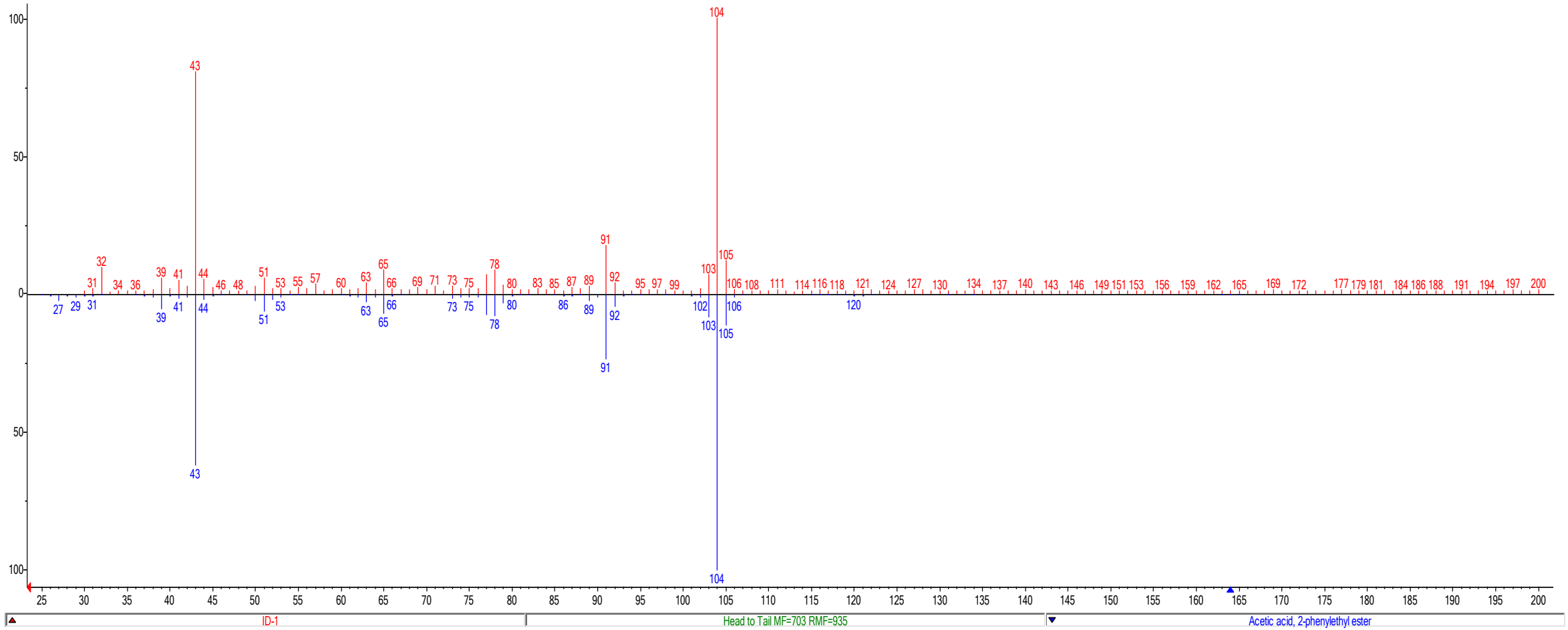
Peak # 104;
RT: 21.953 min
Suggested ID: Octanoic acid



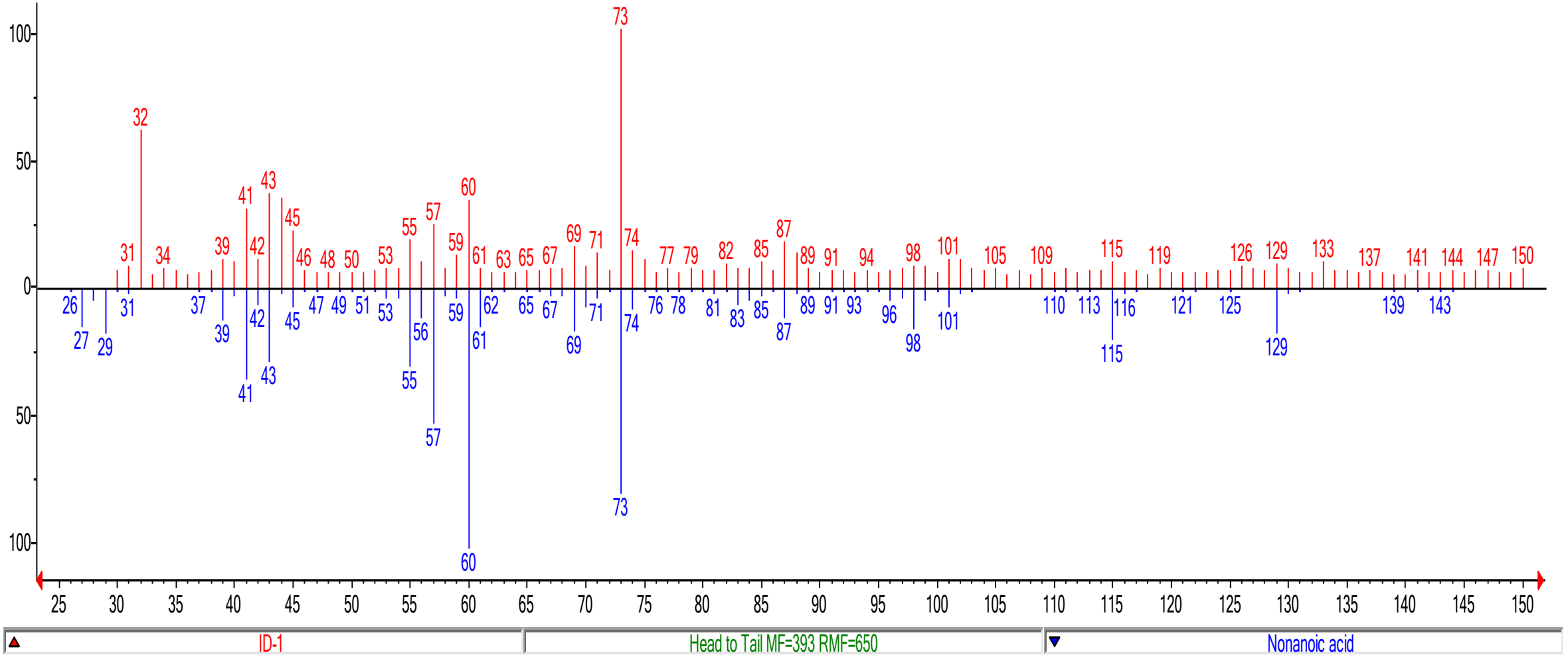
Peak # 106;
RT: 22.561 min
Suggested ID: ethyl octanoate



Peak # 115;
RT: 24.266 min
Suggested ID: 2-phenethyl acetate



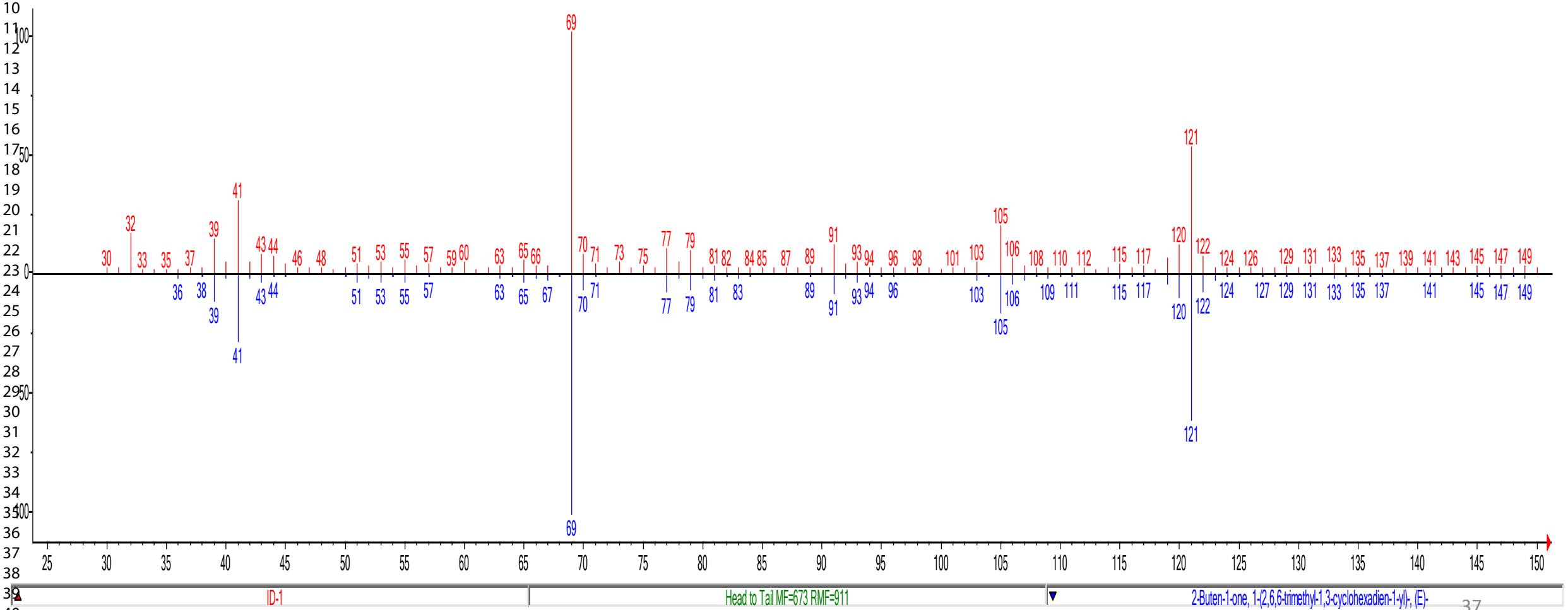
Peak # 124;
RT: 26.249 min
Suggested ID: Nonanoic acid

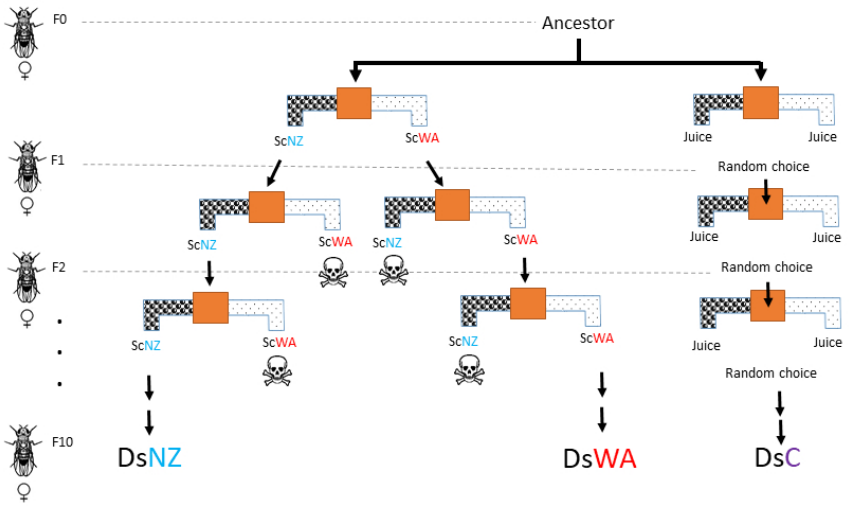


Peak # 129;

RT: 28.952 min

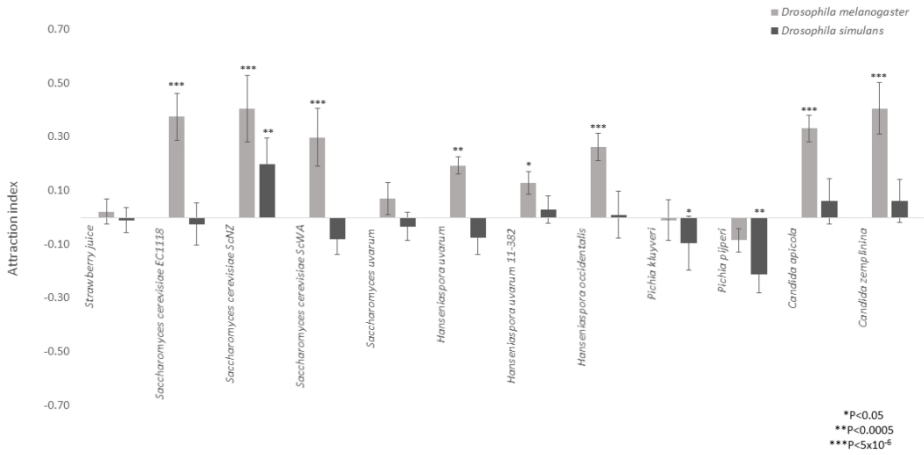
Suggested ID: beta-damascenone





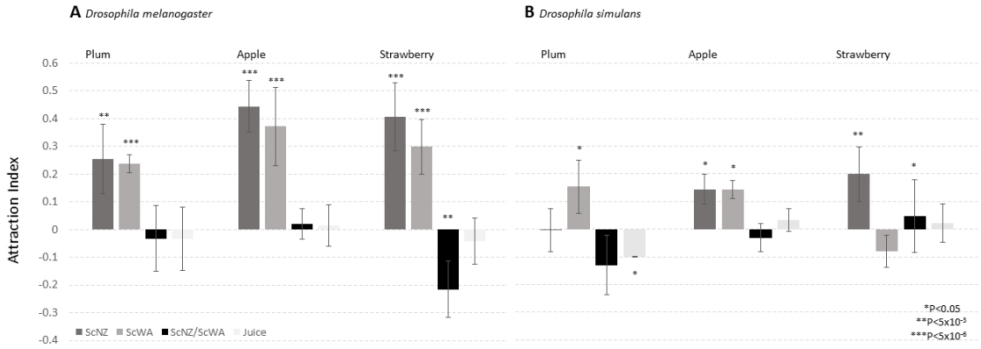
Mechanism of selection for female Drosophila simulans flies (Ds) with preference to an initially attractive Saccharomyces cerevisiae strain (ScNZ) and initially unattractive strain (ScWA) when inoculated in strawberry juice. . Triplicate populations were founded for each treatment from the ancestral population and were evolved over a six month period equating to ten Drosophila generations. The treatments were: 1) control populations (DsC), where no selection for fly choice was applied: the next generation was founded from flies selected from one side of the T-maze at random; 2) selection for flies choosing the yeast strain that was attractive to the founding ancestral fly population (ScNZ) and these are labelled DsNZ; and 3) selection for flies choosing the yeast strain (ScWA) that was significantly less preferred by the founding ancestral fly population, and these populations are labelled DsWA. Selection for fly preference was applied each generation by head-to-head competition between ScNZ and ScWA with 60-80 female flies per replicate population in duplicate. According to choice, 32-70 flies per population were transferred to fresh media for oviposition and killed after three days.

250x150mm (96 x 96 DPI)



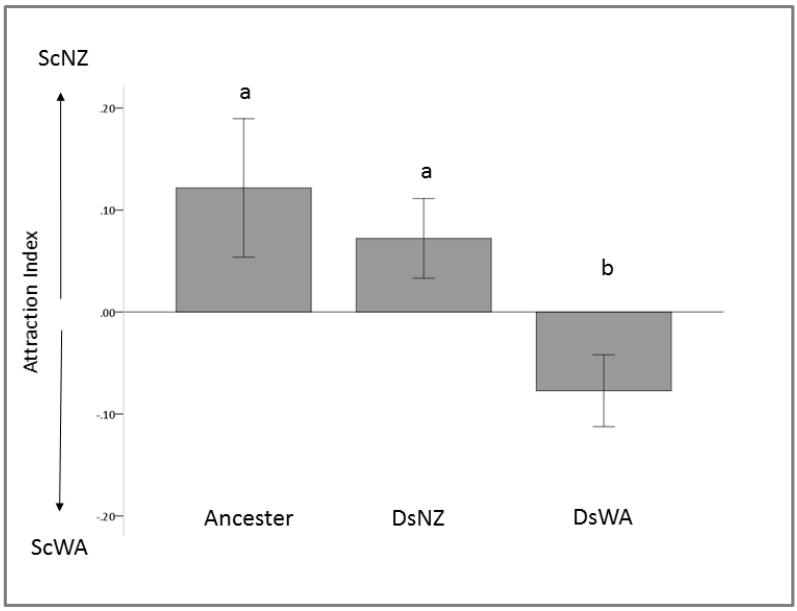
Drosophila melanogaster and D. simulans attraction to a range of Saccharomycetaceae yeasts grown in strawberry juice. Error bars represent the standard error of the mean attraction index and significance in binomial distribution of choice tests is indicated by asterisks (N=6, α=0.05).

338x190mm (96 x 96 DPI)



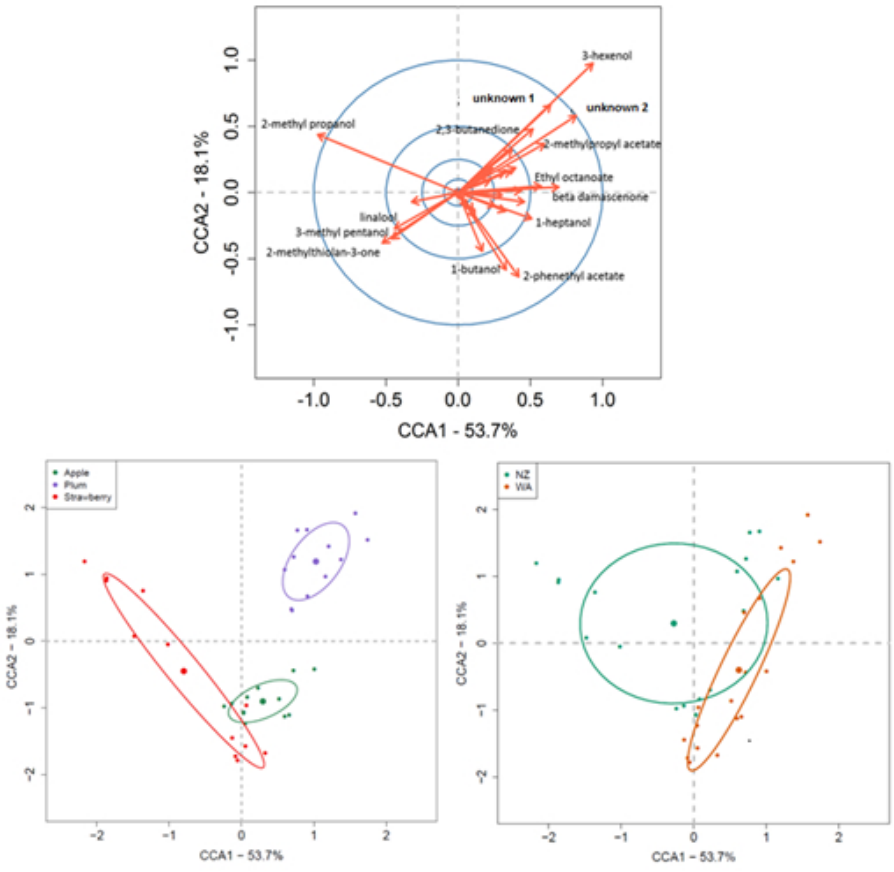
Attractiveness of Saccharomyces cerevisiae strains ScNZ and ScWA to Drosophila melanogaster (A) and D. simulans (B) when inoculated in sterile fruit juice and compared against juice of the same fruit type as control. Binary competition experiments (ScNZ/ScWA) test ferments of both yeasts against each other, where a positive Attraction index (AI) indicates preference for ScNZ and a negative AI preference for ScWA. Error bars represent the standard error of the mean AI and significance in binomial distribution of choice tests is indicated by asterisks (N=6, α=0.05).

338x190mm (96 x 96 DPI)



Change in Drosophila simulans preference to S. cerevisiae isolates ScNZ and ScWA in strawberry juice over ten fly generations. Error bars represent the standard error of the mean attraction index (AI, N=6) for ancestral D. simulans and lines selected for attraction to ScNZ (DsNZ) or ScWA (DsWA), respectively. Positive AI indicate preference for ScNZ and negative AI attraction to ScWA and treatments not connected by the same letter are significantly different (ANOVA, Tukey HSD test at α=0.05).

254x190mm (96 x 96 DPI)



Constrained Correspondence Analysis visualisation of fruit ferments using the chemicals listed in Table 1. (A) The direction and magnitude of all loading vectors, with labels for chemicals that report a magnitude larger than 0.5. The blue circles represent the position of 0.1, 0.25, 0.5 and 1 for reference. (B) Sample points coloured by fruit type with 50% ellipses. (C) Samples point coloured by yeast genotype with 50% ellipses

150x158mm (96 x 96 DPI)

S	A	P	*Detected in fermented juice only*	ScNZ	ScWA	*Increased in fermented juice*
✓ScWA	✓ScWA	✓ScWA	3-hydroxybutanone (Acetoin)°	↑28x**	↑39x**	1-propanol
✓	✓	✓	2,4,5-trimethyl-1,3-dioxolane A°	↑6x**	↑7x**	2-pentanone (Methylpropyl ketone)
✓ScWA	✓ScWA	✓ScWA	2,4,5-trimethyl-1,3-dioxolane B°	↑65x**	↑170x**	ethyl propanoate
✓	✓	✓	2-methylethyl propanoate (Isopropyl propanoate)	↑11000x**	↑8000x**	3-methyl butanol (Isoamyl alcohol)
✓	✓	✓ScNZ	3-methyl-1-pentanol	↑18x**	↑25x**	2-methyl butanol (active Amyl alcohol)
✓	✓	✓	unknown 2	↑3x*	↑5x**	ethyl butanoate
✓	✓	✓	unknown 3	↑2x**	↑3x**	1-hexanol
✓	✓	✓	methyl 2-hydroxy-4-methyl pentanoate	↑29x**	↑25x**	3-methylbutyl acetate (Isoamylacetate)
✓	✓	✓	2-methylthiolan-3-one (Blackberry Thiophenone)	↑2x**	ns	ethyl acetate
✓	✓	✓	1-heptanol	↑56x*	ns	2-methyl-1-propanol (Isobutanol)
✓	✓	✓	2-phenylethanol (Benzeneethanol)	ns	↑72x**	ethyl hexanoate (Ethyl caproate)
✓	✓	✓	ethyl octanoate (Ethyl caprylate)	ns	↑460x*	octanoic acid (Caprylic acid)
✓	✓	✓	2-phenethyl acetate	ns	↑10x**	nonanoic acid (Pelargonic acid)

ScNZ	ScWA	*Reduced in fermented juice*	ScNZ	ScWA	*Unchanged*
↓13x**	↓7x**	1-butanol	ns	ns	unknown 1
↓11x*	↓21x*	**A:** 2-methylbutyl acetate (amyl acetate)	ns	ns	2-methylpropyl acetate (Isobutyl acetate)
↓48x**	↓27x**	1-hexanal	ns	ns	3-hexenol
↓11x*	↓8x*	**P**: 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one (β-damascenone)	ns	ns	hexyl acetate
			ns	ns	3,7-Dimethyl-1,6-octadien-3-ol (Linalool)
			ns	ns	2,3-butanedione (Diacetyl)

Drosophila attraction to yeast in Strawberry

fly species	left	right	flies left	flies right
simulans	Juice	H.uvarum5	24	16
simulans	Juice	S.cerevisiae EC11183	18	14
simulans	Juice	Juice	13	16
simulans	H.uvarum3	Juice	26	28
simulans	Juice	H.uvarum4	14	14
simulans	Juice	S.cerevisiae EC11182	23	24
simulans	H.uvarum1	Juice	16	16
simulans	S.cerevisiae EC11184	Juice	21	27
simulans	Juice	S.cerevisiae EC11181	16	29
simulans	Juice	Juice	18	18
simulans	H.uvarum2	Juice	13	25
simulans	S.cerevisiae EC11185	Juice	15	26
simulans	P.kluyveri4	Juice	27	26
simulans	P.pijperi4	Juice	14	36
simulans	Juice	Juice	19	20
simulans	Juice	P.pijperi1	30	21
simulans	P.pijperi5	Juice	32	33
simulans	Juice	P.kluyveri6	40	17
simulans	P.kluyveri2	Juice	23	37
simulans	Juice	P.pijperi2	30	21
simulans	P.kluyveri1	Juice	23	28
simulans	Juice	P.kluyveri5	12	23
simulans	P.pijperi6	Juice	30	35
simulans	Juice	P.pijperi3	39	17
simulans	Juice	H.uvarum6	20	24
simulans	Juice	Juice	21	36
simulans	P.kluyveri3	Juice	22	31
simulans	Juice	H.occidentalis4	20	39
simulans	Juice	Juice	20	20
simulans	H.occidentalis2	Juice	20	18
simulans	Juice	Juice	19	17
simulans	H.occidentalis1	Juice	13	16
simulans	H.uvarum_11-382-3	Juice	25	23
simulans	Juice	H.uvarum_11-382-1	16	24
simulans	H.uvarum11-382_4	Juice	23	26
simulans	H.occidentalis3	Juice	30	21
simulans	S.cerevisiae EC11186	Juice	30	27
simulans	H.uvarum11-382_2	Juice	27	20
simulans	Juice	C.zemplininia4	10	16
simulans	C.zemplininia2	Juice	22	12
simulans	Juice	C.apicola4	21	28
simulans	C.zemplininia1	Juice	19	18
simulans	Juice	Juice	17	16
simulans	Juice	H.occidentalis5	32	20
simulans	C.apicola1	Juice	16	22
simulans	Juice	C.apicola2	18	17
simulans	Juice	Juice	33	21
simulans	C.apicola5	Juice	15	6
simulans	Juice	H.uvarum11-382_5	17	16

simulans	Juice	H.occidentalis6	20	14
simulans	Juice	Juice	9	15
simulans	Juice	C.zemplininia5	22	13
simulans	H.uvarum11-382_6	Juice	11	14
simulans	Juice	C.apicola7	25	23
simulans	C.zemplinina7	Juice	24	24
simulans	Juice	C.zemplinina6	18	27
simulans	C.apicola6	Juice	25	25
simulans	Juice	Juice	18	20
simulans	Juice	Juice	23	14
simulans	S.uvarum4	Juice	26	27
simulans	Juice	S.uvarum3	28	23
simulans	Juice	Juice	18	12
simulans	Juice	S.uvarum1	21	20
simulans	S.uvarum6	Juice	32	21
simulans	Juice	Juice	31	30
simulans	S.uvarum5	Juice	22	31
simulans	Juice	S.uvarum2	28	23
melanogaster	Juice	Juice	40	22
melanogaster	S.cerevisiae EC11184	Juice	32	19
melanogaster	Juice	H.uvarum5	24	46
melanogaster	H.uvarum3	Juice	46	27
melanogaster	Juice	S.cerevisiae EC11182	17	39
melanogaster	H.uvarum2	Juice	41	26
melanogaster	S.cerevisiae EC11186	Juice	36	22
melanogaster	Juice	H.uvarum6	27	30
melanogaster	H.uvarum1	Juice	43	24
melanogaster	Juice	Juice	27	25
melanogaster	Juice	S.cerevisiae EC11183	25	37
melanogaster	Juice	S.cerevisiae EC11181	19	43
melanogaster	S.cerevisiae EC11185	Juice	41	5
melanogaster	Juice	Juice	28	33
melanogaster	Juice	H.uvarum4	10	24
melanogaster	Juice	P.kluyveri4	23	37
melanogaster	Juice	P.pijperi2	27	30
melanogaster	P.kluyveri1	Juice	27	32
melanogaster	Juice	Juice	22	30
melanogaster	Juice	P.kluyveri5	31	25
melanogaster	Juice	P.pijperi1	31	31
melanogaster	Juice	P.pijperi3	34	26
melanogaster	Juice	P.kluyveri6	25	39
melanogaster	P.pijperi6	Juice	29	33
melanogaster	P.pijperi4	Juice	20	33
melanogaster	P.kluyveri3	Juice	12	16
melanogaster	P.kluyveri2	Juice	18	26
melanogaster	P.pijperi5	Juice	28	36
melanogaster	Juice	Juice	23	30
melanogaster	H.uvarum_11-382-5	Juice	34	24
melanogaster	Juice	Juice	18	32
melanogaster	H.occidentalis2	Juice	38	28
melanogaster	Juice	H.occidentalis4	19	28
melanogaster	Juice	H.occidentalis6	16	47

melanogaster	Juice	Juice	26	28
melanogaster	Juice	H.occidentalis5	22	34
melanogaster	Juice	H.uvarum_11-382-1	32	28
melanogaster	H.occidentalis1	Juice	34	18
melanogaster	Juice	H.uvarum_11-382-3	24	32
melanogaster	H.uvarum_11-382-4	Juice	25	16
melanogaster	H.uvarum_11-382-6	Juice	31	24
melanogaster	H.occidentalis3	Juice	37	24
melanogaster	Juice	H.uvarum_11-382-2	26	37
melanogaster	C.apicola6	Juice	39	18
melanogaster	Juice	C.zemplininia6	9	41
melanogaster	Juice	C.apicola1	15	24
melanogaster	C.zemplininia2	Juice	37	23
melanogaster	Juice	C.apicola4	16	45
melanogaster	Juice	C.zemplininia5	22	47
melanogaster	Juice	C.zemplininia7	9	45
melanogaster	Juice	Juice	42	20
melanogaster	C.zemplininia4	Juice	44	18
melanogaster	C.zemplininia1	Juice	29	26
melanogaster	C.apicola5	Juice	47	19
melanogaster	Juice	C.apicola2	24	32
melanogaster	C.apicola7	Juice	39	19
melanogaster	S.uvarum5	Juice	34	27
melanogaster	Juice	Juice	27	30
melanogaster	Juice	S.uvarum4	21	39
melanogaster	Juice	S.uvarum3	29	24
melanogaster	Juice	S.uvarum2	25	30
melanogaster	S.uvarum6	Juice	23	19
melanogaster	Juice	Juice	21	21
melanogaster	Juice	Juice	14	13
melanogaster	Juice	S.uvarum1	26	22

Host fruit context of Drosophila attraction to ScNZ and ScWA

fly species	fruit	Yeast	left	right	flies left	flies right
simulans	Strawberry	None	Juice	Juice	25	16
simulans	Strawberry	None	Juice	Juice	13	12
simulans	Strawberry	None	Juice	Juice	19	33
simulans	Strawberry	None	Juice	Juice	20	22
simulans	Strawberry	None	Juice	Juice	22	20
simulans	Strawberry	None	Juice	Juice	36	27
simulans	Strawberry	both	ScNZA	ScWAA	7	17
simulans	Strawberry	both	ScNZD	ScWAC	21	10
simulans	Strawberry	both	ScWAB	ScNZC	31	35
simulans	Strawberry	both	ScWAD	ScNZF	16	25
simulans	Strawberry	both	ScNZE	ScWAF	34	14
simulans	Strawberry	both	ScWAE	ScNZB	25	30
simulans	Strawberry	ScNZ	Juice	ScNZA	14	15
simulans	Strawberry	ScNZ	ScNZD	Juice	19	15
simulans	Strawberry	ScNZ	ScNZC	Juice	33	24
simulans	Strawberry	ScNZ	Juice	ScNZF	10	46
simulans	Strawberry	ScNZ	Juice	ScNZE	19	33
simulans	Strawberry	ScNZ	ScNZB	Juice	32	34
simulans	Strawberry	ScWA	ScWAA	Juice	11	13
simulans	Strawberry	ScWA	Juice	ScWAC	16	10
simulans	Strawberry	ScWA	Juice	ScWAB	32	25
simulans	Strawberry	ScWA	ScWAD	Juice	23	34
simulans	Strawberry	ScWA	ScWAF	Juice	28	29
simulans	Strawberry	ScWA	Juice	ScWAE	26	36
melanogaster	Strawberry	None	Juice	Juice	23	18
melanogaster	Strawberry	None	Juice	Juice	33	23
melanogaster	Strawberry	None	Juice	Juice	28	26
melanogaster	Strawberry	None	Juice	Juice	6	13
melanogaster	Strawberry	None	Juice	Juice	31	28
melanogaster	Strawberry	None	Juice	Juice	21	37
melanogaster	Strawberry	both	ScNZD	ScWAF	33	24
melanogaster	Strawberry	both	ScNZE	ScWAG	24	19
melanogaster	Strawberry	both	ScWAA	ScNZC	46	20
melanogaster	Strawberry	both	ScWAC	ScNZF	37	16
melanogaster	Strawberry	both	ScNZG	ScWAC	31	40
melanogaster	Strawberry	both	ScWAE	ScNZB	38	17
melanogaster	Strawberry	ScNZ	Juice	ScNZD	38	34
melanogaster	Strawberry	ScNZ	Juice	ScNZE	18	29
melanogaster	Strawberry	ScNZ	ScNZC	Juice	41	14
melanogaster	Strawberry	ScNZ	ScNZF	Juice	35	1
melanogaster	Strawberry	ScNZ	Juice	ScNZG	24	53
melanogaster	Strawberry	ScNZ	ScNZB	Juice	49	19
melanogaster	Strawberry	ScWA	ScWAF	Juice	46	18
melanogaster	Strawberry	ScWA	ScWAG	Juice	37	22
melanogaster	Strawberry	ScWA	Juice	ScWAB	38	24
melanogaster	Strawberry	ScWA	Juice	ScWAA	40	6
melanogaster	Strawberry	ScWA	ScWAC	Juice	40	28
melanogaster	Strawberry	ScWA	Juice	ScWAE	35	32
simulans	apple	None	Juice	Juice	24	27

simulans	apple	None	Juice	Juice	35	28
simulans	apple	None	Juice	Juice	14	11
simulans	apple	None	Juice	Juice	28	23
simulans	apple	None	Juice	Juice	25	23
simulans	apple	None	Juice	Juice	23	29
simulans	apple	both	ScWAF	ScNZE	24	24
simulans	apple	both	ScWAB	ScNZD	29	24
simulans	apple	both	ScNZB	ScWAC	23	18
simulans	apple	both	ScNZC	ScWAA	28	28
simulans	apple	both	ScWAG	ScNZG	33	20
simulans	apple	both	ScNZF	ScWAE	32	30
simulans	apple	ScNZ	ScNZE	Juice	31	24
simulans	apple	ScNZ	Juice	ScNZD	14	32
simulans	apple	ScNZ	Juice	Juice	23	21
simulans	apple	ScNZ	ScNZC	Juice	30	21
simulans	apple	ScNZ	Juice	ScNZG	24	26
simulans	apple	ScNZ	Juice	ScNZF	23	27
simulans	apple	ScWA	Juice	ScWAF	25	32
simulans	apple	ScWA	Juice	ScWAB	12	21
simulans	apple	ScWA	ScWAC	Juice	28	20
simulans	apple	ScWA	ScWAA	Juice	17	16
simulans	apple	ScWA	ScWAG	Juice	32	24
simulans	apple	ScWA	Juice	ScWAE	26	33
melanogaster	apple	None	Juice	Juice	12	13
melanogaster	apple	None	Juice	Juice	33	24
melanogaster	apple	None	Juice	Juice	13	20
melanogaster	apple	None	Juice	Juice	36	30
melanogaster	apple	None	Juice	Juice	30	45
melanogaster	apple	None	Juice	Juice	40	22
melanogaster	apple	both	ScNZE	ScWAC	36	30
melanogaster	apple	both	ScWAF	ScNZD	29	25
melanogaster	apple	both	ScNZC	ScWAA	40	30
melanogaster	apple	both	ScWAB	ScNZB	31	28
melanogaster	apple	both	ScNZG	ScWAD	27	39
melanogaster	apple	both	ScWAG	ScNZF	23	34
melanogaster	apple	ScNZ	Juice	ScNZE	20	45
melanogaster	apple	ScNZ	ScNZD	Juice	48	12
melanogaster	apple	ScNZ	Juice	ScNZC	6	48
melanogaster	apple	ScNZ	ScNZB	Juice	44	16
melanogaster	apple	ScNZ	Juice	ScNZG	20	47
melanogaster	apple	ScNZ	ScNZF	Juice	32	30
melanogaster	apple	ScWA	ScWAC	Juice	51	8
melanogaster	apple	ScWA	Juice	ScWAF	11	66
melanogaster	apple	ScWA	ScWAE	Juice	21	28
melanogaster	apple	ScWA	Juice	ScWAB	13	60
melanogaster	apple	ScWA	ScWAA	Juice	34	31
melanogaster	apple	ScWA	Juice	ScWAG	27	44
simulans	plum	None	Juice	Juice	31	21
simulans	plum	None	Juice	Juice	19	26
simulans	plum	None	Juice	Juice	38	26
simulans	plum	None	Juice	Juice	20	26
simulans	plum	None	Juice	Juice	16	15

simulans	plum	None	Juice	Juice	18	22
simulans	plum	both	ScWAF	ScNZF	27	30
simulans	plum	both	ScWAB	ScNZE	52	12
simulans	plum	both	ScNZB	ScWAA	29	27
simulans	plum	both	ScWAC	ScNZG	21	21
simulans	plum	both	ScNZD	ScWAB	21	22
simulans	plum	both	ScWAD	ScNZA	28	18
simulans	plum	ScNZ	Juice	ScNZF	25	25
simulans	plum	ScNZ	Juice	ScNZD	24	18
simulans	plum	ScNZ	ScNZE	Juice	19	29
simulans	plum	ScNZ	ScNZG	Juice	26	24
simulans	plum	ScNZ	ScNZB	Juice	22	24
simulans	plum	ScNZ	Juice	ScNZA	18	36
simulans	plum	ScWA	ScWAG	Juice	20	15
simulans	plum	ScWA	Juice	ScWAE	31	35
simulans	plum	ScWA	Juice	ScWAC	23	30
simulans	plum	ScWA	ScWAG	Juice	16	17
simulans	plum	ScWA	Juice	ScWAB	21	25
simulans	plum	ScWA	ScWAD	Juice	10	38
melanogaster	plum	None	Juice	Juice	13	30
melanogaster	plum	None	Juice	Juice	55	19
melanogaster	plum	None	Juice	Juice	17	22
melanogaster	plum	None	Juice	Juice	10	14
melanogaster	plum	None	Juice	Juice	28	37
melanogaster	plum	None	Juice	Juice	32	24
melanogaster	plum	both	ScWAG	ScNZE	45	23
melanogaster	plum	both	ScNZB	ScWAC	21	26
melanogaster	plum	both	ScWAD	ScNZG	20	16
melanogaster	plum	both	ScNZA	ScWAA	45	14
melanogaster	plum	both	ScWAE	ScNZF	25	36
melanogaster	plum	both	ScNZD	ScWAB	22	38
melanogaster	plum	ScNZ	ScNZE	Juice	28	23
melanogaster	plum	ScNZ	Juice	ScNZB	22	35
melanogaster	plum	ScNZ	ScNZG	Juice	49	9
melanogaster	plum	ScNZ	Juice	ScNZA	6	25
melanogaster	plum	ScNZ	ScNZF	Juice	28	39
melanogaster	plum	ScNZ	Juice	ScNZD	32	36
melanogaster	plum	ScWA	Juice	ScWAG	34	41
melanogaster	plum	ScWA	ScWAC	Juice	41	22
melanogaster	plum	ScWA	Juice	ScWAF	30	44
melanogaster	plum	ScWA	ScWAA	Juice	44	22
melanogaster	plum	ScWA	Juice	ScWAD	24	40
melanogaster	plum	ScWA	ScWAB	Juice	20	12

competition experiments

		No flies preference	
Generation	fly line	ScNZ	ScWA
F0	Ancestor	7	17
F0	Ancestor	21	10
F0	Ancestor	35	31
F0	Ancestor	25	16
F0	Ancestor	34	14
F0	Ancestor	30	25
F0	Ancestor	22	31
F0	Ancestor	23	18
F0	Ancestor	31	20
F0	Ancestor	30	23
F0	Ancestor	24	21
F0	Ancestor	35	16
F11	DsC1	28	22
F11	DsC1	34	19
F11	DsC1	32	30
F11	DsC1	28	29
F11	DsC1	27	24
F11	DsC1	18	11
F11	DsC2	27	20
F11	DsC2	22	26
F11	DsC2	25	17
F11	DsC2	23	24
F11	DsC2	32	26
F11	DsC2	21	35
F11	DsC3	27	20
F11	DsC3	26	33
F11	DsC3	35	25
F11	DsC3	30	14
F11	DsC3	28	19
F11	DsC3	35	17
F11	DsNZ1	30	23
F11	DsNZ1	24	15
F11	DsNZ1	21	29
F11	DsNZ1	29	24
F11	DsNZ1	27	17
F11	DsNZ1	24	16
F11	DsNZ2	23	22
F11	DsNZ2	25	34
F11	DsNZ2	32	16
F11	DsNZ2	19	31
F11	DsNZ2	18	19
F11	DsNZ2	22	26
F11	DsNZ3	27	30
F11	DsNZ3	30	18
F11	DsNZ3	23	20
F11	DsNZ3	25	24
F11	DsNZ3	23	14
F11	DsNZ3	18	12
F11	DsWA1	29	29

F11	DsWA1	22	32	
F11	DsWA1	26	25	
F11	DsWA1	24	33	
F11	DsWA1	23	22	
F11	DsWA1	21	28	
F11	DsWA2	26	31	
F11	DsWA2	19	33	
F11	DsWA2	28	27	
F11	DsWA2	27	34	
F11	DsWA2	15	15	
F11	DsWA2	10	22	
F11	DsWA3	9	18	
F11	DsWA3	23	15	
F11	DsWA3	26	23	
F11	DsWA3	11	13	
F11	DsWA3	19	20	
F11	DsWA3	18	16	
Generation	fly line	Juice	Juice	
F0	Ancestor	25	16	
F0	Ancestor	13	12	
F0	Ancestor	19	33	
F0	Ancestor	20	22	
F0	Ancestor	22	20	
F0	Ancestor	36	27	
F0	Ancestor	8	20	
F0	Ancestor	19	19	
F0	Ancestor	25	23	
F11	mix	25	21	
F11	mix	33	29	
F11	mix	28	29	
F11	mix	28	25	
F11	mix	32	35	
F11	mix	18	17	

			Standardised peak area	
Name	**Retention t**	**Peak #**	**ScNZSA**	**ScNZSG**
1-Propanol	**1.829**	**P1**	1.060	1.147
2,3-Butanedione	**1.919**	**P2**	0.224	0.142
Formic acid, 1-methylpropyl ester	**2.06**	**P3**	0.000	0.000
Furan, 2-methyl-	**2.103**	**P4**	0.000	0.000
Ethyl Acetate	**2.133**	**P5**	7.229	5.221
1-Propanol, 2-methyl-	**2.292**	**P6**	10.239	11.042
Butanal, 3-methyl-	**2.453**	**P7**	0.000	0.000
Butanal, 2-methyl-	**2.566**	**P8**	0.000	0.000
1-Butanol	**2.637**	**P9**	0.051	0.042
1-Penten-3-one	**2.794**	**P10**	0.000	0.000
2-Pentanone	**2.817**	**P11**	0.112	0.051
1-Penten-3-ol	**2.851**	**P12**	0.000	0.000
2,3-Pentanedione	**2.896**	**P13**	0.014	0.005
Pentanal	**2.919**	**P14**	0.000	0.000
Carbonic acid, ethyl-, methyl ester	**3.042**	**P15**	0.224	0.068
2-Butanone, 3-hydroxy-	**3.107**	**P16**	0.000	0.000
Propanoic acid, ethyl ester	**3.256**	**P17**	0.364	0.000
Butanoic acid, methyl ester	**3.437**	**P18**	0.100	0.000
1,3-Dioxolane, 2,4,5-trimethyl-	**3.581**	**P19**	0.468	0.253
1-Butanol, 3-methyl-	**3.96**	**P20**	113.535	91.122
2-methyl butanol	**3.99**	**P21**	7.408	4.244
1,3-Dioxolane, 2,4,5-trimethyl-	**4.109**	**P22**	0.000	0.000
2-Butenoic acid, methyl ester, (E)-	**4.156**	**P23**	0.000	0.000
Acetic acid, 1-methylpropyl ester	**4.22**	**P24**	0.000	0.000
Propanoic acid, 2-methyl-, ethyl ester	**4.274**	**P25**	0.234	0.074
1-Pentanol	**4.483**	**P26**	0.033	0.024
Acetic acid, 2-methylpropyl ester	**4.573**	**P27**	0.091	0.038
2,3-Butanediol, [R-(R*,R*)]-	**4.714**	**P28**	0.000	0.046
Butanoic acid, 2-methyl-, methyl ester, (.+/-.)	**4.654**	**P29**	0.000	0.000
Hexanal	**5.038**	**P30**	0.000	0.000
Butanoic acid, ethyl ester	**5.265**	**P31**	0.331	0.116
Propanoic acid, 2-methyl-	**5.173**	**P32**	0.772	0.000
Acetic acid, butyl ester	**5.615**	**P33**	0.000	0.000
Pentanoic acid, methyl ester	**5.926**	**P34**	0.000	0.000
Acetyl valeryl	**6.188**	**P35**	0.000	0.000
2-Hexenal, (E)-	**6.293**	**P36**	0.000	0.000
1-Pentanol, 4-methyl-	**6.462**	**P37**	0.049	0.058
2-Hexenal, (E)-	**6.714**	**P38**	0.000	0.000
1-Pentanol, 3-methyl-	**6.721**	**P39**	0.137	0.076
3-Hexanol, 2-methyl-	6.784	**P40**	0.000	0.000
1-Propanol, 3-ethoxy-	6.719	**P41**	0.000	0.000
3-Hexenol	**7.022**	**P42**	0.011	0.024
2-Hexen-1-ol, (E)-	**7.448**	**P43**	0.000	0.000
1-Hexanol	**7.627**	**P44**	6.325	3.142
1-Butanol, 3-methyl-, acetate	**7.784**	**P45**	0.617	0.265
1-Butanol, 2-methyl-, acetate	**7.892**	**P46**	0.000	0.000
Propanal, 3-(methylthio)-	**7.943**	**P47**	0.000	0.000
Butanoic acid, 2-methyl-	**7.967**	**P48**	0.000	0.000
2-Heptanone	**8.087**	**P49**	0.000	0.000

3-methyl 3-Hexanol	8.122	**P50**	0.000	0.000
Pentanoic acid, 4-methyl-, methyl ester	**8.324**	**P51**	0.000	0.000
Heptanal	**8.467**	**P52**	0.000	0.000
Propanoic acid, butyl ester	**9.075**	**P53**	0.000	0.000
Butanoic acid, 2-methyl-	**9.101**	**P54**	0.000	0.000
Acetic acid, pentyl ester	**9.282**	**P55**	0.000	0.000
2-Penten-1-ol, acetate, (Z)-	**9.55**	**P56**	0.000	0.000
Pentanoic acid	**9.373**	**P57**	0.000	0.000
2-Buten-1-ol, 3-methyl-, acetate	**9.547**	**P58**	0.000	0.000
Hexanoic acid, methyl ester	**9.703**	**P59**	0.000	0.000
5-methyl heptanone	**9.86**	**P60**	0.000	0.000
4-Heptanone, 2-methyl-/Octanone	**10.146**	**P61**	0.183	0.093
2-hydroxy-4-methyl, methyl pentanotae	**10.31**	**P62**	0.044	0.022
Butanoic acid, 2-methyl-, propyl ester	**10.755**	**P63**	0.000	0.000
Propanoic acid, 2-methyl-, butyl ester	**11.075**	**P64**	0.000	0.000
3(2H)-Thiophenone, dihydro-2-methyl-	**11.256**	**P65**	0.056	0.032
4-Butoxy-2-butanone	**11.388**	**P66**	0.107	0.038
3-(methylthio)propanol	**11.489**	**P67**	0.015	0.000
3-methylbutyl propanoate	**11.743**	**P68**	0.012	0.000
1-Heptanol	**11.83**	**P69**	0.057	0.027
Benzoic acid, 2-formyl-4,6-dimethoxy-, 8,8-di	**12.054**	**P70**	0.000	0.000
5-Hepten-2-one, 6-methyl-	**12.176**	**P71**	0.000	0.000
1-Octen-3-ol	**12.273**	**P72**	0.000	0.000
7-Octen-2-ol	**12.71**	**P73**	0.000	0.000
dl-6-Methyl-5-hepten-2-ol	**12.848**	**P74**	0.000	0.000
Butanoic acid, butyl ester	**12.942**	**P75**	0.000	0.000
hexyl butanoate	**12.937**	**P76**	0.000	0.000
Hexanoic acid, ethyl ester	**13.107**	**P77**	0.557	0.285
3-Hexen-1-ol, acetate, (Z)-	**13.342**	**P78**	0.000	0.000
3-Eicosyne	**13.503**	**P79**	0.000	0.000
hexyl acetate	**13.747**	**P80**	0.041	0.010
Benzeneacetaldehyde	**14.084**	**P81**	0.000	0.000
1-Hexanol, 2-ethyl-	**14.681**	**P82**	0.000	10.999
ethyl 2-Hexenoate	**14.957**	**P83**	0.000	0.000
Hexanoic acid, 1-methylethyl ester	**15.02**	**P84**	0.000	0.000
Butyl 2-methylbutanoate	**15.212**	**P85**	0.000	0.000
3(2H)-Furanone, 4-methoxy-2,5-dimethyl-	**15.313**	**P86**	0.286	0.168
cis-linalool oxide	**16.485**	**P87**	0.049	0.000
Octanol	**16.631**	**P88**	0.000	0.000
2,5-Dimethyl-4-hydroxy-3(2H)-furanone (furan	**16.749**	**P89**	0.000	0.000
Propanoic acid, 2-methyl-, propyl ester	17.076	**P90**	0.000	0.000
trans Linalool oxide	**17.174**	**P91**	0.224	0.142
Butanoic acid,3-hexen-1-yl ester	**17.267**	**P92**	0.000	0.000
Heptanoic acid	**17.72**	**P93**	0.000	0.000
Phenylethyl Alcohol	**17.76**	**P94**	3.724	1.591
Nonanal	**17.803**	**P95**	0.000	0.000
LINALOOL	**17.903**	**P96**	1.230	0.734
2-Butoxyethyl acetate	**18.207**	**P97**	0.000	0.000
Butanoic acid, 3-hydroxy-, butyl ester	**18.94**	**P98**	0.000	0.000
Hexanoic acid, 2-ethyl-	**19.56**	**P99**	0.000	0.000
phenylmethyl acetate	**20.13**	**P100**	0.000	0.000
6-ethenyltetrahydro-2,2,6-trimethyl-2H-Pyra	**21.159**	**P101**	0.000	0.000

1-Nonanol	21.363	**P102**	0.000	0.000
p-menth-1-en-8-ol	**21.846**	**P103**	0.117	0.000
Octanoic Acid	**21.953**	**P104**	0.000	0.234
Butanoic acid, hexyl ester	**22.329**	**P105**	0.000	0.000
ethyl octanoate	**22.561**	**P106**	0.000	0.000
Decanal	**22.658**	**P107**	0.000	0.000
1H-Indene, 2,3-dihydro-1,1,5,6-tetramethyl-	23.133	**P108**	0.000	0.000
octyl acetate	**23.181**	**P109**	0.000	0.000
1-Heptanol, 2-propyl-	**23.268**	**P110**	0.000	0.000
2(3H)-Furanone, 5-butyldihydro-	**23.67**	**P111**	0.000	0.000
cis-geraniol	23.73	**P112**	0.000	0.000
beta-citronellol	23.804	**P113**	0.000	0.000
1-Octanol, 2,7-dimethyl- (dehydrocitronellol)	**24.116**	**P114**	0.000	0.000
2-phenethyl acetate	**24.266**	**P115**	0.008	0.000
Butanoic acid, 2-methyl-, hexyl ester	**24.327**	**P116**	0.000	0.000
transgeraniol	**24.807**	**P117**	0.000	0.000
Benzene, 2-(2-butenyl)-1,3,5-trimethyl-	**24.792**	**P118**	0.000	0.000
Benzene, 1,3-bis(1,1-dimethylethyl)-	**24.987**	**P119**	0.000	0.000
Hexanoic acid, 2-ethyl-, methyl ester	**25.103**	**P120**	0.000	0.000
(1,4-Dimethylpent-2-enyl)benzene	**25.33**	**P121**	0.000	0.000
1-Decanol	**25.626**	**P122**	0.000	0.000
2-Penten-1-ol, 2-methyl-5-(2-methyl-3-methy	**25.893**	**P123**	0.000	0.000
Nonanoic acid	26.249	**P124**	2.586	0.346
1-Octanol, 2-butyl-	**27.115**	**P125**	0.000	0.000
Butanoic acid, 3-hydroxy-, butyl ester	**27.287**	**P126**	0.000	0.000
Decanoic acid, methyl ester	**27.413**	**P127**	0.000	0.000
1-Decanol, 2-methyl-	**27.578**	**P128**	0.000	0.000
beta damascenone	28.952	**P129**	0.000	0.000

ScNZSB	ScNZSF	ScNZSD	ScNZSA	ScWASA	ScWASB	Strawberry ScWASC	ScWASD
1.350	1.268	1.279	2.646	0.904	0.000	1.474	1.038
0.235	0.080	0.028	0.208	0.107	0.085	0.014	0.202
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5.130	6.220	9.460	12.549	4.300	3.091	2.789	1.821
0.000	11.912	14.710	0.000	1.509	1.240	0.363	0.342
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.026	0.063	0.018	0.052	0.071	0.000	0.093	0.072
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.073	0.054	0.076	0.080	0.103	0.099	0.057	0.074
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.331	0.073	0.268	0.190	0.231	0.219	0.129	0.164
0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.065
0.190	0.000	0.215	0.189	0.274	0.392	0.353	0.304
0.000	0.015	0.080	0.000	0.022	0.000	0.023	0.049
0.199	0.372	0.300	0.363	0.541	0.000	0.749	0.536
123.754	99.003	126.313	204.290	23.371	63.093	4.000	57.724
10.619	9.741	9.927	15.487	4.449	5.510	55.676	7.505
0.000	0.000	0.000	0.000	0.011	0.000	0.060	0.009
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.167	0.170	0.222	0.286	0.030	0.050	0.071	0.065
0.033	0.000	0.000	0.000	0.017	0.000	0.013	0.030
0.145	0.000	0.086	0.000	0.017	0.032	0.040	0.051
0.000	0.527	0.000	0.000	0.000	0.150	0.000	0.252
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.278	0.140	0.205	0.205	0.346	0.270	0.216	0.368
0.758	0.318	0.778	1.515	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.063	0.022	0.083	0.085	0.000	0.000	0.035	0.020
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.100	0.078	0.131	0.226	0.065	0.020	0.102	0.129
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.011	0.000	0.028	0.000	0.015	0.000	0.015	0.015
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5.493	3.410	6.253	5.941	5.061	5.691	4.153	5.912
1.087	0.531	0.902	1.097	0.335	0.223	0.267	0.463
0.110	0.356	0.245	0.377	0.162	0.040	0.040	0.171
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.058	0.000	0.329	0.451
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.088	0.021	0.038	0.000	0.080	0.071	0.058	0.000
0.000	0.000	0.000	0.000	0.011	0.000	0.012	0.000
0.108	0.088	0.111	0.000	0.104	0.000	0.057	0.082
0.029	0.029	0.039	0.042	0.017	0.000	0.014	0.016
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.080	0.033	0.067	0.073	0.009	0.000	0.018	0.016
0.080	0.053	0.093	0.110	0.100	0.000	0.076	0.101
0.021	0.000	0.007	0.000	0.000	0.000	0.000	0.000
0.016	0.000	0.010	0.000	0.000	0.000	0.000	0.000
0.042	0.024	0.061	0.043	0.215	0.171	0.095	0.193
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.138	0.704	0.647	0.891	1.585	1.603	0.914	1.623
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.049	0.019	0.038	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	12.634	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.255	0.159	0.281	0.246	0.143	0.000	0.160	0.253
0.033	0.025	0.049	0.048	0.034	0.042	0.038	0.054
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.283	0.138	0.239	0.268	0.260	0.509	0.193	0.257
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3.012	1.846	3.108	2.000	0.819	1.311	0.635	1.397
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.197	0.728	1.328	1.210	1.154	1.063	0.867	1.228
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.287	0.000	0.000	0.041	0.147	0.234	0.000	0.174
0.211	0.000	0.475	0.000	1.213	0.645	0.366	1.056
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.021	0.000	0.013	0.000	0.085	0.027	0.025	0.047
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.009	0.000	0.009	0.000	0.026	0.039	0.012	0.017
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.545	1.445	1.960	0.000	1.730	0.125	0.000	0.902
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

ScWASE	ScWASF	JuiceS1	JuiceS2	JuiceS3	JuiceS4	JuiceS5	JuiceS6
1.164	1.554	0.029	0.007	0.001	0.001	0.001	0.001
0.243	0.046	1.567	0.946	0.959	0.775	0.189	0.155
0.000	0.000	0.001	0.023	0.000	0.000	0.000	0.023
0.000	0.000	0.000	0.025	0.000	0.002	0.000	0.025
2.155	2.352	0.428	0.306	0.299	0.238	0.156	0.150
0.388	0.355	0.142	0.130	0.107	0.081	0.057	0.056
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.062	0.101	0.926	0.730	0.632	0.434	0.227	0.211
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.054	0.090	0.021	0.015	0.019	0.017	0.014	0.018
0.000	0.000	0.079	0.060	0.061	0.054	0.034	0.031
0.000	0.000	0.000	0.017	0.015	0.013	0.000	0.002
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.128	0.279	0.112	0.074	0.079	0.075	0.059	0.063
0.324	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.385	0.102	0.000	0.000	0.000	0.012	0.004	0.005
0.048	0.114	1.648	1.294	1.362	1.413	0.977	0.991
0.796	0.635	0.000	0.000	0.000	0.000	0.000	0.000
45.092	81.919	0.000	0.007	0.009	0.018	0.015	0.020
3.824	5.820	0.000	0.000	0.000	0.000	0.000	0.000
0.024	0.015	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.035	0.039	0.041	0.041	0.035	0.036
0.000	0.000	0.000	0.012	0.015	0.019	0.013	0.015
0.059	0.343	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.026	0.000	0.000	0.059	0.016	0.012	0.016
0.000	0.086	0.000	0.038	0.053	0.018	0.010	0.012
0.345	0.461	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.034	0.060	0.056	0.036	0.027	0.031
0.000	0.000	3.250	2.671	2.893	2.947	2.207	2.236
0.234	0.455	0.043	0.042	0.047	0.053	0.042	0.048
0.000	0.000	0.068	0.093	0.041	0.000	0.000	0.000
0.000	0.000	0.070	0.057	0.069	0.076	0.063	0.070
0.000	0.000	0.000	0.000	0.002	0.037	0.011	0.013
0.000	0.000	0.000	0.013	0.011	0.215	0.081	0.081
0.000	0.000	0.126	0.100	0.123	0.338	0.253	0.267
0.000	0.044	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	9.206	7.070	7.826	8.574	7.247	7.930
0.051	0.136	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.018	0.000	0.000	0.022	0.027	0.017	0.024
0.000	0.000	0.140	0.125	0.193	0.202	0.178	0.204
4.171	6.547	0.133	0.121	0.272	0.254	0.197	0.208
0.269	0.762	0.031	0.052	0.150	0.106	0.070	0.072
0.047	0.275	0.000	0.000	0.012	0.012	0.013	0.014
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.432	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.310	0.141	0.077	0.070

0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.013	0.000	0.110	0.090
0.000	0.000	0.000	0.012	0.014	0.000	0.089	0.087
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.172	0.366
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.014	0.017
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004
0.053	0.105	1.296	1.082	1.230	1.344	1.225	1.471
0.000	0.026	0.000	0.000	0.000	0.000	0.000	0.011
0.090	0.094	0.000	0.000	0.000	0.000	0.000	0.000
0.017	0.020	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.009	0.023	0.000	0.000	0.000	0.000	0.000	0.000
0.079	0.150	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.144	0.183	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.047	0.007	0.006
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.221	1.857	1.191	0.000	0.406	0.144	0.071	0.077
0.000	0.000	0.000	0.000	0.000	0.166	0.061	0.056
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.141	0.000	1.669	0.670	0.333	0.338
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.047	0.037	0.042	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.246	0.283	0.255	0.271	0.357	0.466	2.058	2.290
0.037	0.060	0.048	0.036	0.046	0.051	0.090	0.050
0.000	0.000	0.000	0.000	0.012	0.010	0.004	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.245	0.281	0.310	0.263	0.280	0.255	0.216	0.245
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.024	0.015	0.000
0.630	1.491	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.710	1.412	2.603	2.431	2.394	2.226	1.559	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.067	0.000	0.061	0.025	0.015	0.016
0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.004
0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.006

0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.118	0.186	0.183	0.230	0.191	0.199	0.150	0.127
0.537	0.750	0.000	0.000	0.000	0.014	0.010	0.013
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.033	0.043	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.013	0.005	0.006
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.014	0.008	0.013
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.018	0.022	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.003	0.007	0.005
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.229	0.410	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

ScNZPLA	ScNZPLB	ScNZPLD	ScNZPLE	ScNZPLF	ScNZPLG	ScWAPLA	ScWAPLB
1.370	1.650	1.602	2.470	1.180	1.511	3.311	2.488
0.216	0.000	0.173	0.012	0.237	0.000	0.000	0.813
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.315	1.746	1.475	2.068	1.213	1.321	3.338	2.069
2.115	1.650	1.938	0.000	1.971	0.000	0.785	1.440
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.026	0.000	0.000	0.037	0.057	0.039	0.057	0.059
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.041	0.070	0.054	0.044	0.027	0.029	0.000	0.040
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.074	0.057	0.041	0.041	0.000	0.018	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.286	0.265	0.331	0.276	0.452	0.000	0.418	0.194
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.116
0.176	0.261	0.237	0.270	0.132	0.218	1.081	0.336
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.191	0.000	0.000	0.018	0.000	0.070	0.000	1.456
58.329	66.121	78.452	89.790	66.017	58.618	91.918	48.977
4.214	4.071	3.873	0.000	3.915	0.000	9.527	6.978
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.066
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.203	0.193	0.135	0.237	0.102	0.227	0.557	0.178
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.284	0.426	0.443	0.522	0.382	0.377	0.492	0.336
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.277	0.298	0.280	0.000	0.230	0.000	0.000	0.000
0.517	0.438	0.468	0.000	0.438	0.351	0.219	0.363
0.351	0.248	0.000	0.331	0.237	0.415	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.019	0.000	0.000	0.026	0.021	0.000	0.000	0.000
0.034	0.056	0.000	0.083	0.000	0.000	0.104	0.079
0.000	0.000	0.000	0.269	0.133	0.146	0.198	0.104
1.441	1.404	1.470	1.464	1.417	1.288	1.712	1.664
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6.261	6.097	7.799	6.712	6.499	5.514	8.342	7.407
0.767	1.149	1.223	1.433	1.157	0.906	0.743	0.541
0.177	0.263	0.244	0.327	0.235	0.136	0.203	0.133
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.170	0.131	0.048	0.000	0.132	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.131	0.054	0.058	0.063	0.083	0.074	0.080	0.085
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.042	0.050	0.000	0.052	0.046	0.041	0.140	0.282
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.386	0.399	0.368	0.335	0.459	0.412	0.841	0.687
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.032	0.035	0.033	0.037	0.034	0.031	0.043
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.116	5.329	0.072	0.000	6.021	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.045	0.000	0.000	0.055	0.031	0.069	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6.529	6.676	5.881	5.295	8.732	6.644	3.653	4.164
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.216	0.134	0.173	0.000	0.246	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000	0.036	0.043	0.046	0.077	0.000	0.035	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.323	0.000	0.374	0.000	0.321	0.149	1.477	2.369
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.029	0.031	0.000	0.115	0.117
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.078	0.151	0.000	0.166	0.095	0.067	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.041
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.020	0.024	0.000	0.020	0.035	0.027	0.025	0.043
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.044	0.000	0.042	0.000	0.037	0.046	0.000	0.063
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.790	0.000	1.205	1.243	0.000	1.244	4.441	4.628
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.047	0.039	0.050	0.030	0.057	0.038	0.030	0.042

Plum ScWAPLC	ScWAPLD	ScWAPLE	ScWAPLF 0.	JuicePLA	JuicePLB	JuicePLC	JuicePLD
1.878	2.710	3.039	3.127	0.092	0.000	0.029	0.017
0.000	0.000	0.222	0.000	0.034	0.027	0.016	0.021
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.891	2.099	2.143	2.442	3.199	2.270	2.412	2.738
0.640	0.827	1.650	1.471	0.017	0.000	0.005	0.005
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.110	0.000	0.086	0.000	0.168	0.122	0.130	0.112
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.102	0.060	0.063	0.067	0.000	0.009	0.015	0.011
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.031	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.017	0.015	0.017	0.015
0.224	0.265	0.792	0.455	0.710	0.653	0.724	0.806
0.398	0.304	0.000	0.000	0.000	0.000	0.000	0.000
0.625	0.778	0.432	0.579	0.000	0.000	0.000	0.009
0.000	0.000	0.000	0.000	0.000	0.016	0.017	0.000
2.202	1.733	0.913	0.000	0.000	0.000	0.000	0.000
36.807	51.995	69.956	86.226	0.029	0.015	0.000	0.000
5.673	6.316	0.000	9.931	0.000	0.028	0.046	0.025
0.056	0.042	0.096	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.100	0.200	0.257	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.212	0.461	0.411	0.455	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.504	0.597	0.727	6.172	5.398	6.150	5.969
0.000	0.158	0.888	0.638	0.165	0.148	0.170	0.175
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.013	0.017	0.030
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.815	0.955	1.021	1.079
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.042	0.000	0.060	0.000	0.000	0.000	0.000
0.091	0.000	0.183	0.000	0.000	0.000	0.000	0.000
1.462	1.484	1.826	1.598	1.989	1.782	2.466	2.606
0.000	0.000	0.000	0.000	0.082	0.086	0.109	0.000
7.315	7.300	8.466	8.613	4.715	4.689	5.362	5.814
0.343	0.538	0.608	0.640	0.000	0.000	0.000	0.000
0.184	0.301	0.160	0.428	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.052	0.055	0.000	0.052	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.047	0.000	0.020	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.093	0.071	0.086	0.073	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.232	0.231	0.291	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.058	0.019	0.016	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.018
0.000	0.000	0.000	0.000	0.000	0.000	0.046	0.047
0.526	0.651	0.726	0.723	0.000	0.000	0.055	0.046
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.018	0.016	0.009
0.024	0.036	0.050	0.028	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.294	0.510	0.465	0.403
0.000	0.000	0.000	0.000	0.000	9.001	5.504	2.486
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.027	0.030	0.032
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.057	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.369	2.550	4.045	2.798	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.108	0.099	0.000	0.029	0.160	0.000	0.138	0.107
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000	0.029	0.032	0.056	0.367	0.378	0.320	0.215
0.147	0.000	0.141	0.000	0.000	0.164	0.131	0.093
1.114	1.357	1.714	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.124	0.154	0.159	0.098	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.068	0.052	0.026	0.025
0.113	0.000	0.158	0.226	0.186	0.124	0.133	0.064
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.141	0.141	0.127	0.094
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.023	0.021	0.026	0.000	0.000	0.000	0.000	0.000
0.071	0.017	0.020	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.153	0.174	0.145	0.094
0.023	0.022	0.042	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.053	0.000	0.069	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.059	0.039	0.029	0.025
0.000	0.000	0.000	0.000	0.222	0.121	0.059	0.027
0.000	0.000	0.000	0.000	0.086	0.075	0.026	0.012
0.000	0.000	0.000	0.000	0.000	0.016	0.013	0.012
0.000	0.000	0.000	0.000	0.571	0.683	0.464	0.281
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.390	3.298	5.306	2.061	0.000	2.192	0.943	0.299
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.015	0.012	0.004
0.000	0.000	0.000	0.000	0.000	0.000	0.061	0.036
0.040	0.035	0.079	0.106	0.000	0.000	1.523	0.493

JuicePLE	JuicePLF	ScNZJGB	ScNZJGC	ScNZJGD	ScNZJGE	ScNZJGF	ScNZJGG
0.010	0.011	0.636	0.729	0.552	0.552	0.545	0.484
0.010	0.011	0.038	0.106	0.090	0.038	0.070	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.262	0.000	1.732	5.340	2.063	1.830	0.000	0.000
0.006	0.000	0.437	0.000	0.431	0.455	0.551	0.477
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.049	0.062	0.259	0.196	0.163	0.234	0.155	0.143
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.004	0.006	0.030	0.041	0.030	0.033	0.024	0.034
0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.011	0.009	0.021	0.021	0.000	0.000
0.016	0.016	0.000	0.000	0.000	0.000	0.000	0.000
0.586	0.499	0.081	0.069	0.072	0.076	0.069	0.067
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.002	0.003	0.088	0.099	0.093	0.196	0.122	0.112
0.014	0.015	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.029	0.000	0.028	0.049	0.035	0.000
0.005	0.006	82.211	74.924	89.091	72.950	61.354	61.696
0.008	0.010	7.819	10.003	6.691	7.269	6.964	6.257
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.059	0.123	0.127	0.089	0.091	0.116
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.102	0.065	0.105	0.079	0.076	0.066
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4.603	4.018	0.000	0.000	0.000	0.000	0.000	0.000
0.142	0.161	0.074	0.060	0.071	0.084	0.069	0.064
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.118	0.071	0.103	0.097	0.045	0.048
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.018	0.017	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.079	0.935	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.019	0.026	0.022	0.018	0.014	0.024
0.000	0.000	0.000	0.027	0.016	0.024	0.026	0.018
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.210	1.868	0.059	0.061	0.064	0.062	0.039	0.067
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4.870	4.234	4.954	4.103	4.837	4.524	3.568	3.777
0.000	0.000	0.500	0.384	0.588	0.506	0.438	0.324
0.000	0.000	0.193	0.300	0.292	0.273	0.210	0.094
0.024	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.015	0.013	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.040	0.104	0.000	0.052	0.066	0.082
0.000	0.000	0.018	0.015	0.023	0.022	0.020	0.014
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.031	0.013	0.032	0.019	0.021	0.016
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.025	0.028	0.047	0.036	0.033	0.018
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.016	0.020	0.026	0.044	0.027	0.036
0.017	0.014	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.026	0.060	0.000	0.000	0.000	0.000	0.000	0.000
0.026	0.010	0.000	0.000	0.000	0.000	0.000	0.000
0.044	0.032	0.269	0.433	0.480	0.492	0.470	0.674
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.014	0.024	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.015	0.012	0.027	0.018	0.016	0.018
0.506	0.343	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.065	0.089	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.008	0.014	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	2.064	4.286	3.703	3.334	1.708	4.381
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.163	0.128	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.223	0.143	0.000	0.000	0.000	0.000	0.000	0.000
0.074	0.067	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.255	0.402	0.542	0.000	0.000	0.330
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.019	0.014	0.026	0.025	0.028	0.015
0.026	0.017	0.000	0.000	0.000	0.000	0.000	0.000
0.102	0.105	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.080	0.046	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.087	0.046	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.014	0.023	0.022	0.028	0.021
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.026	0.027	0.000	0.000	0.000	0.000	0.000	0.000
0.015	0.005	0.000	0.000	0.000	0.000	0.000	0.000
0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.009	0.007	0.000	0.000	0.000	0.000	0.000	0.000
0.275	0.145	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000
0.151	0.000	0.000	1.060	1.105	2.025	1.876	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.013	0.018	0.000	0.000	0.000	0.000	0.000	0.000
0.036	0.025	0.000	0.000	0.000	0.000	0.000	0.000
0.289	0.000	0.009	0.000	0.019	0.009	0.000	0.025

Apple

ScWAJGA	ScWAJGB	ScWAJGC	ScWAJGE	ScWAJGF	ScWAJGG	JuiceJGA	JuiceJGB
2.475	0.988	1.127	1.098	1.204	1.302	0.093	0.259
0.002	0.172	0.000	0.075	0.145	0.000	0.018	0.078
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.959	1.323	2.298	1.207	1.923	0.000	0.033	0.111
1.176	0.578	1.077	0.688	0.960	0.550	0.141	0.211
0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.011
0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.006
0.662	0.191	0.327	0.203	0.288	0.367	3.472	5.014
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.121	0.065	0.086	0.069	0.000	0.059	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.032	0.009	0.019	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.155	0.048	0.216	0.082	0.138	0.121	0.000	0.006
0.000	0.053	0.026	0.000	0.116	0.000	0.000	0.000
0.816	0.352	0.693	0.412	0.536	0.431	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.307	0.971	0.093	1.069	1.195	0.083	0.000	0.000
93.178	72.761	95.839	66.593	98.910	106.984	0.000	0.000
15.255	6.461	9.151	6.462	9.902	8.978	1.556	1.707
0.000	0.033	0.000	0.028	0.056	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.245	0.207	0.165	0.151	0.209	0.088	0.000	0.000
0.000	0.000	0.033	0.000	0.035	0.033	0.029	0.049
0.447	0.165	0.310	0.000	0.325	0.175	0.380	0.402
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	1.463	1.693
0.360	0.162	0.376	0.204	0.263	0.195	0.000	0.000
0.000	0.214	0.199	0.152	0.281	0.000	0.000	0.000
0.145	0.000	0.230	0.083	0.184	0.142	7.999	8.795
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.064	0.070
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	4.800	5.696
0.081	0.081	0.049	0.050	0.062	0.049	0.000	0.000
0.026	0.020	0.028	0.028	0.039	0.021	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.161	0.116	0.133	0.119	0.147	0.096	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.060
11.139	5.871	9.408	8.327	10.824	7.320	1.143	1.459
1.750	0.610	1.050	0.870	1.105	0.748	0.000	0.000
0.674	0.000	0.447	0.298	0.421	0.364	5.132	5.172
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.146	0.000

0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.038
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.148
0.000	0.000	0.000	0.000	0.000	0.000	0.137	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.028	0.000	0.023	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.200	0.147	0.147	0.178	0.164	0.102	0.000	0.000
0.060	0.029	0.059	0.029	0.042	0.035	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.085	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.052	0.000	0.052	0.000	0.023	0.043	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.278	0.218	0.247	0.225	0.252	0.071	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	2.356	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.039	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.139
0.000	0.000	0.000	0.000	0.000	0.000	0.184	0.076
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.096	1.311	1.360	1.685	2.037	0.697	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.032	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.137	0.106	0.147	0.168	0.214	0.000	0.000	2.276
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.033	0.000	0.019	0.000	0.074	0.000	0.000	0.000
0.000	0.011	0.009	0.012	0.015	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.044
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5.382	5.158	5.187	5.566	5.882	2.853	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.078	0.051
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.062	0.025
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.097
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.170	0.704	1.219	0.000	1.250	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.098	0.072	0.106	0.115	0.146	0.051	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.287	0.058
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.084	0.000	0.000
0.294	0.344	0.281	0.417	0.439	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.246
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.583	3.110	1.325	2.766	0.508	0.426	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.028
0.000	0.000	0.000	0.000	0.000	0.000	0.043	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.030	0.054	0.178	0.155	0.053	0.000	0.000	0.000

JuiceJGC	JuiceJGD	JuiceJGE	JuiceJGF
0.145	0.059	0.021	0.016
0.033	0.008	0.012	0.001
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.078	0.042	0.026	0.024
0.143	0.069	0.059	0.048
0.008	0.003	0.002	0.002
0.005	0.003	0.002	0.002
4.200	2.252	1.897	1.450
0.000	0.019	0.014	0.007
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.003
0.000	0.000	0.000	0.000
0.000	0.006	0.003	0.003
0.004	0.002	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
1.410	0.871	0.736	0.573
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.043	0.038	0.033	0.027
0.460	0.375	0.312	0.263
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
1.968	1.942	1.705	1.543
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
6.346	2.486	1.965	1.531
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.082	0.094	0.094	0.097
0.000	0.000	0.000	0.000
5.021	5.264	5.368	5.504
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.013	0.013	0.020
0.062	0.087	0.087	0.084
1.405	1.379	1.375	1.444
0.000	0.000	0.000	0.000
6.244	4.332	3.458	3.933
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000

0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.009	0.011	0.010	0.017
0.048	0.057	0.051	0.054
0.000	0.000	0.000	0.000
0.189	0.225	0.203	0.217
0.000	0.007	0.006	0.003
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.004	0.005	0.000
0.000	0.010	0.009	0.010
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.011	0.011	0.011
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.022	0.020	0.019
0.000	0.009	0.009	0.010
0.000	0.000	0.000	0.000
0.132	0.143	0.144	0.160
0.093	0.115	0.096	0.114
0.000	0.000	0.000	0.000
0.000	0.003	0.001	0.002
0.000	0.011	0.007	0.009
0.000	0.000	0.000	0.000
2.836	2.958	2.712	2.934
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.059	0.056	0.054	0.055
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.005	0.006	0.014
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.018	0.017	0.015	0.014
0.000	0.000	0.000	0.000
0.000	0.010	0.010	0.013
0.034	0.005	0.003	0.005
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000

0.114	0.117	0.123	0.134
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.012	0.019	0.017
0.000	0.000	0.000	0.000
0.039	0.039	0.041	0.004
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.086	0.080	0.041	0.042
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.009	0.010	0.013
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.004
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.346	0.319	0.308	0.317
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.044	0.042	0.053	0.054
0.000	0.005	0.007	0.010
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000